RESOLUTION OENO 2/96

DETERMINATION METHOD OF THE ISOTOPIC RATIO 18O/16O OF THE WATER CONTENT IN WINES

THE GENERAL ASSEMBLY,

IN VIEW of article 5, paragraph 4 of the International Convention for the Unification of Methods of Analysis and Appreciation of Wine of 13 October 1954,

ON THE PROPOSAL of the Sub-Commission of the Methods of Analysis and Wine Appreciation,

DECIDES:

To include the following method in Appendix A of the Compendium of International Methods of Wine Analysis:

Determination method for isotopic ratio 18O/16O of water content in wines - Description of the method and inter-laboratory study

I. Description of the method

1. Method objective:

The objective of the present method is to measure the isotopic ratio 18O/16O of waters of different origines. The isotopic ratio 18O/16O can be expressed in deviation ▯‰ in ratio to the value of isotopic ratio of the international reference V.SMOW :

$$\delta_i[\%] = \left[\frac{R_i}{R_{SMOW}} - 1\right] \times 1000$$

2. Principle:

The isotopic ratio 18O/16O is determined by mass spectrometry of isotopic ratios (MSIR) from ionic currents m/z 46 ($^{12}C^{16}O^{18}O$) et 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 :

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$$C^{16}O_2 + H_2{}^{18}O \ \square \ C^{16}O^{18}O + H_2{}^{16}O$$

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 standardised 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 CO2 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. Manuel 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 adapt ther 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.

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 stabilisation 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) during 12 hours (one night). To optimise the necessary time for equilibration, it is advised to prepare the samples at the end of the day and let the balance set itself during the night.

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, let the measuring cells 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 5

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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 phial's is then transferred into the introduction chamber of the mass spectrometer by a capillary tube and the measure 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 (I46,I44) between the sample and the reference is expressed in ‰ by the means of the following relation:

$$\delta_{'} sample = \left[\frac{(I_{46}/I_{44})sample}{(I_{46}/I_{44})reference} - 1\right] \times 1000$$

The 18O content of the sample compared to the reference V.SMOW on the V. SMOW-SLAP scale, is given by the relation :

$$\delta'_{18O} = \left[\frac{\delta'_{sample} - \delta'_{SMOW}}{\delta'_{SMOW} - \delta'_{SLAP}}\right] \times 55{,}5$$

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 ten measures on unknown samples.

7. Fidelity :

- the repeatability (r) is equal to 0.24‰
- the reproducibility (R) is equal to 0.50‰

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13. **SLAP**

Standard Light Antartic Precipitation
Disponible à l'Agence Internationale de l'Energie Atomique, Vienne, Autriche

14. **V.SMOW**

Vienna Standard Mean Ocean Water
Disponible à l'Agence Internationale de l'Energie Atomique, Vienne, Autriche

ANNEX

Not to be published in the Compendium but will have to be published in the O.I.V. Bulletin

II. COLLABORATIVE INTER-LABORATORY STUDY:

1. Participants:

The inter-laboratory study was carried out with the participation of fourteen laboratories from Germany, France, Italy, the Netherlands, United Kingdom and Switzerland.

2. Samples:

Samples 1 and 2: Local tap water (2 identical samples)
Sample

- 3: White wine A
- 4: Red wine B + 20% water
- 5: Red wine B
- 6: White wine A + 3% water
- 7: White wine A + 7% water
- 8: Red wine B + 5% water
- 9: Red wine B + 10% water
- 10: White wine A + 15% water

3. Number of iterations:

Three determinations were carried out on each sample.

4. Statistical evaluation of the results corresponding to the ISO 5725:1986 standard

4.1. Maximum variance test

The Cochran test is used to determine if one laboratory's variance measures is significantly greater than an other laboratory.

4.2. Test on the average:

The Dixon test in two parts allows to verify if the weakest or strongest average can be considered as abnormal.

5. Results:

The results are shown in the adjoining tables. In laboratories 8, 11 and 12 several abnormal values appeared which have been excluded from repeatability r and from reproducibility R calculation.

SAMPLE 1

Lab. N°	1	2	3	n	□	s	S^2
1	-8,29	-8,33	-8,36	3	-8,324	0,0356	0,0013
2	-7,96	-7,67	-7,82	3	-7,817	0,1465	0,0215
*3	-8,64	-8,24	-8,76	3	-8,546	0,2697	0,0727
4	-8,29	-8,22	-8,30	3	-8,270	0,0436	0,0019
5	-8,27	-8,13	-8,26	3	-8,220	0,0781	0,0061
6	-8,20	-8,21	-8,23	3	-8,213	0,0153	0,0002
7	-8,25	-8,23	-8,30	3	-8,260	0,0361	0,0013
*8	-8,61	-8,47	-8,52	3	-8,531	0,0726	0,0053

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9	-8,29	-8,29	-8,31	3	-8,295	0,0103	0,0001
10	-8,25	-8,24	-8,40	3	-8,297	0,0856	0,0073
*11	-9,12	-9,19	-9,40	3	-9,237	0,1457	0,0212
*12	-7,64	-7,82	-7,97	3	-7,810	0,1652	0,0273
13	-7,96	-8,06	-7,97	3	-7,999	0,0541	0,0029
14	-8,30	-8,20	-8,30	3	-8,267	0,0577	0,0033

□ = -8,2‰

s_r = 0,068‰

r = 0,19‰

s_R = 0,171‰

R = 0,48‰

SAMPLE 2

Lab. N°	1	2	3	n	□	s	S^2
1	-8,37	-8,33	-8,38	3	-8,359	0,0257	0,0007
2	-7,85	-8,16	-8,08	3	-8,032	0,1600	0,0256
3	-8,29	-7,96	-8,30	3	-8,184	0,1943	0,0378
4	-8,34	-8,30	-8,28	3	-8,307	0,0306	0,0009
5	-8,26	-8,19	-8,22	3	-8,223	0,0351	0,0012
6	-8,34	-8,26	-8,31	3	-8,303	0,0404	0,0016
7	-8,22	-8,29	-8,29	3	-8,267	0,0404	0,0016
*8	-8,35	-8,59	-8,51	3	-8,485	0,1246	0,0155
9	-8,29	-8,30	-8,16	3	-8,252	0,0766	0,0059

10	-8,27	-8,28	-8,03	3	-8,196	0,1412	0,0199
*11	-9,13	-9,31	-9,26	3	-9,233	0,0929	0,0086
*12	-7,57	-7,90	-8,30	3	-7,923	0,3656	0,1336
13	-7,97	-8,07	-7,98	3	-8,002	0,0544	0,0030
14	-8,20	-8,30	-8,30	3	-8,267	0,0577	0,0033

□ = -8,22‰

s_r = 0,096‰

r = 0,27‰

s_R = 0,136‰

R = 0,38‰

SAMPLE 3

Lab. N°	1	2	3	n	□	s	s2
1	-1,69	-1,61	-1,56	3	-1,622	0,0661	0,0044
2	-1,49	-1,30	-1,51	3	-1,433	0,1175	0,0138
3	-1,78	-1,69	-1,55	3	-1,674	0,1120	0,0125
4	-1,52	-1,49	-1,41	3	-1,473	0,0569	0,0032
5	-1,47	-1,43	-1,48	3	-1,460	0,0265	0,0007
6	-1,33	-1,26	-1,22	3	-1,270	0,0557	0,0031
7	-1,72	-1,76	-1,71	3	-1,730	0,0265	0,0007
*8	-1,98	-1,87	-1,53	3	-1,791	0,2336	0,0546
9	-1,54	-1,54	-1,45	3	-1,510	0,0557	0,0031
10	-1,64	-1,60	-1,60	3	-1,612	0,0269	0,0007
*11	-3,64	-2,74	-3,12	3	3,167	0,4518	0,2041

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*12	-1,50	-1,95	-2,13	3	-1,860	0,3245	0,1053
13	-1,33	-1;40	-1,36	3	-1,362	0,0384	0,0015
14	-1,80	-1,80	-1,70	3	-1,767	0,0577	0,0033

□ = 1,54‰

s_r = 0,065‰

r = 0,18‰

s_R = 0,165‰

R = 0,46‰

SAMPLE 4

Lab. N°	1	2	3	n	□	s	S^2
1	3,37	3,51	3,29	3	3,388	0,1077	0,0116
2	3,82	3,64	4,07	3	3,845	0,2185	0,0477
3	3,67	3,98	3,92	3	3,856	0,1627	0,0265
4	3,59	3,64	3,63	3	3,620	0,0265	0,0007
5	3,71	3,78	3,74	3	3,743	0,0351	0,0012
6	3,46	3,33	3,37	3	3,387	0,0666	0,0044
7	3,30	3,36	3,42	3	3,360	0,0600	0,0036
*8	3,18	3,29	3,22	3	3,231	0,0564	0,0032
9	3,51	3,54	3,56	3	3,538	0,0260	0,0007
10	3,57	3,39	3,61	3	3,523	0,1149	0,0132
*11	2,00	1,93	2,07	3	2,000	0,0700	0,0049
*12	2,69	2,76	2,60	3	2,683	0,0802	0,0064
13	3,80	3,63	3,78	3	3,739	0,0918	0,0084

14	3,50	3,40	3,40	3	3,433	0,0577	0,0033

 = 3,59‰

s_r = 0,106‰

r = 0,30‰

s_R = 0,205‰

R = 0,57‰

SAMPLE 5

Lab. N°	1	2	3	n		s	S^2
1	6,60	6,72	6,59	3	6,635	0,0748	0,0056
2	7,18	7,04	7,33	3	7,179	0,1445	0,0209
3	6,98	7,18	6,80	3	6,986	0,1895	0,0359
4	7,00	7,03	7,08	3	7,037	0,0404	0,0016
5	7,05	7,11	7,09	3	7,083	0,0306	0,0009
6	6,69	6,70	6,63	3	6,673	0,0379	0,0014
7	6,48	6,60	6,55	3	6,543	0,0603	0,0036
*8	6,57	6,61	6,49	3	6,553	0,0616	0,0038
9	6,96	6,92	6,90	3	6,925	0,0295	0,0009
10	6,87	6,74	6,84	3	6,816	0,0690	0,0048
*11	5,06	4,76	5,24	3	5,020	0,2425	0,0588
*12	5,87	5,61	5,29	3	5,590	0,2905	0,0844
13	7,04	6,88	6,99	3	6,970	0,0841	0,0071
14	6,90	6,60	6,70	3	6,733	0,1528	0,0233

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□ = 6,87‰

s_r = 0,098‰

r = 0,27‰

s_R = 0,220‰

R = 0,62‰

SAMPLE 6

Lab. N°	1	2	3	n	□	s	s^2
1	-1,87	-1,79	-1,84	3	-1,831	0,0407	0,0017
2	-1,67	-1,50	-1,41	3	-1,525	0,1293	0,0167
3	-1,75	-1,68	-1,86	3	-1,762	0,0905	0,0082
4	-1,84	-1,80	-1,76	3	-1,800	0,0400	0,0016
5	-1,65	-1,65	-1,75	3	-1,683	0,0577	0,0033
6	-1,70	-1,74	-1,64	3	-1,693	0,0503	0,0025
7	-1,93	-1,85	-1,96	3	-1,913	0,0569	0,0032
*8	-3,58	-3,45	-3,11	3	-3,380	0,2449	0,0600
9	-1,84	-1,77	-1,83	3	-1,812	0,0332	0,0011
10	-1,81	-2,02	-1,79	3	-1,871	0,1300	0,0169
*11	-3,42	-2,97	-2,76	3	-3,050	0,3372	0,1137
*12	-2,39	-2,63	-2,81	3	-2,610	0,2107	0,0444
13	-1,76	-1,80	-1,76	3	-1,775	0,0241	0,0006
14	-2,00	-2,10	-1,90	3	-2,000	0,1000	0,0100

□ = 1,79‰

s_r = 0,078‰

r = 0,22‰

s_R = 0,141‰

R = 0,40‰

SAMPLE 7

Lab. N°	1	2	3	n	□	s	S^2
1	-1,90	-1,94	-1,95	3	-1,929	0,0229	0,0005
2	-1,94	-1,79	-1,69	3	-1,803	0,1275	0,0163
3	-2,00	-2,04	-2,22	3	-2,086	0,1199	0,0144
4	-2,08	-2,04	-1,94	3	-2,020	0,0721	0,0052
5	-1,94	-1,88	-1,91	3	-1,910	0,0300	0,0009
6	-2,07	-2,06	-2,18	3	-2,103	0,0666	0,0044
7	-2,21	-2,28	-2,19	3	-2,227	0,0473	0,0022
*8	-3,23	-3,74	-3,28	3	-3,417	0,2773	0,0769
9	-2,08	-2,06	-2,10	3	-2,081	0,0206	0,0004
10	-2,10	-2,19	-2,02	3	-2,104	0,0820	0,0067
*11	-3,55	-3,66	-3,18	3	-3,463	0,2515	0,0632
*12	-2,59	-2,86	-3,06	3	-2,837	0,2359	0,0556
13	-1,70	-2,00	-1,75	3	-1,818	0,1599	0,0256
14	-2,20	-2,40	-2,30	3	-2,300	0,1000	0,0100

□ = 2,04‰

s_r = 0,089‰

r = 0,25‰

s_R = 0,173‰

R = 0,49‰

SAMPLE 8

Lab. N°	1	2	3	n	◻	s	s2
1	5,80	5,96	5,93	3	5,896	0,0876	0,0077
2	6,50	6,28	6,45	3	6,410	0,1155	0,0133
3	6,02	6,20	6,07	3	6,097	0,0926	0,0086
4	5,85	6,02	5,98	3	5,950	0,0889	0,0079
5	6,04	6,15	6,20	3	6,130	0,0819	0,0067
6	5,97	6,01	5,99	3	5,990	0,0200	0,0004
7	5,88	5,89	5,93	3	5,900	0,0265	0,0007
*8	5,70	5,07	5,49	3	5,418	0,3191	0,1019
9	6,09	6,05	6,14	3	6,091	0,0458	0,0021
10	6,01	5,83	6,03	3	5,960	0,1107	0,0123
*11	4,50	4,66	4,57	3	4,577	0,0802	0,0064
*12	5,22	4,90	4,60	3	4,907	0,3101	0,0961
13	5,93	5,86	5,92	3	5,905	0,0409	0,0017
14	5,90	5,90	5,90	3	5,900	0,0000	0,0000

◻ = 6,02‰

s_r = 0,074‰

r = 0,21‰

s_R = 0,167‰

R = 0,47‰

SAMPLE 9

Lab. N°	1	2	3	n	◻	s	s2
1	5,10	4,98	5,14	3	5,070	0,0823	0,0068

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2	5,65	5,38	5,66	3	5,560	0,1603	0,0257
3	5,33	5,11	5,07	3	5,171	0,1407	0,0198
4	5,19	5,23	5,28	3	5,233	0,0451	0,0020
5	5,40	5,45	5,39	3	5,413	0,0321	0,0010
6	5,17	5,21	5,14	3	5,173	0,0351	0,0012
7	4,93	4,87	4,88	3	4,893	0,0321	0,0010
*8	4,30	4,65	4,38	3	4,441	0,1807	0,0326
9	5,00	5,19	5,02	3	5,071	0,1069	0,0114
10	5,28	5,14	5,23	3	5,215	0,0728	0,0053
*11	3,86	3,75	3,81	3	3,807	0,0551	0,0030
*12	4,71	4,14	4,10	3	4,317	0,3412	0,1164
13	5,30	5,07	5,23	3	5,197	0,1176	0,0138
14	5,10	5,20	5,00	3	5,100	0,1000	0,0100

□ = 5,19‰

s_r = 0,094‰

r = 0,26‰

s_R = 0,194‰

R = 0,54‰

SAMPLE 10

Lab. N°	1	2	3	n	□	s	s2
1	-2,53	-2,68	-2,51	3	-2,571	0,0912	0,0083
2	-2,15	-2,53	-2,22	3	-2,301	0,2019	0,0408
3	-2,55	-2,52	-2,63	3	-2,567	0,0604	0,0036

4	-2,64	-2,53	-2,52	3	-2,563	0,0666	0,0044
5	-2,20	-2,37	-2,43	3	-2,333	0,1193	0,0142
6	-2,78	-2,65	-2,75	3	-2,727	0,0681	0,0046
7	-2,75	-2,87	-2,85	3	-2,823	0,0643	0,0041
* 8	-3,58	-3,40	-2,90	3	-3,295	0,3527	0,1244
9	-2,82	-2,74	-2,81	3	-2,789	0,0464	0,0021
10	-2,91	-2,76	-2,61	3	-2,761	0,1520	0,0231
*11	-3,99	-4,00	-4,02	3	4,003	0,0153	0,0002
*12	-2,21	-2,43	-2,51	3	2,383	0,1553	0,0241
13	-2,43	-2,60	-2,46	3	2,498	0,0904	0,0082
14	-2,80	-2,80	-2,70	3	2,767	0,0577	0,0033

□ = 2,61‰

s_r = 0,103‰

r = 0,29‰

s_R = 0,200‰

R = 0,56‰

Summary of statistical results

	□	s_r	r	s_R	R
Water					
Sample 1	-8,20	0,068	0,19	0,171	0,48
Sample 2	-8,22	0,096	0,27	0,136	0,38
Wine N°1					

Sample 5	6,87	0,098	0,27	0,220	0,62
Sample 8	6,02	0,074	0,21	0,167	0,47
Sample 9	5,19	0,0094	0,26	0,194	0,54
Sample 4	3,59	0,106	0,30	0,205	0,57
Wine N°2					
Sample 3	-1,54	0,065	0,18	0,165	0,46
Sample 6	-1,79	0,078	0,22	0,141	0,40
Sample 7	-2,04	0,089	0,25	0,173	0,49
Sample 10	-2,61	0,103	0,29	0,200	0,56

□ : general average (‰)
s_r : standard deviation of repeatability (‰)
r : repeatability (‰)
S_R: standard deviation of reproducibility (‰)
R : reproducibility (‰).

Certified in conformity Cape Town, 15th November 1996
The Director General of the OIV
Secretary of the General Assembly
Robert TINLOT