Method OIV-MA-AS312-03A Type II method

Methanol

(Resolution Oeno 377/2009, Revised by OIV-OENO 480/2014)

1. Scope of application

This method is applicable to the determination of methanol in wine for concentrations between 50 and 500 mg/L.

2. Principle

Methanol is determined in the distillate, to which ethanol is an internal standard is added, using gas chromatography with a flame ionisation detector (FID).

3. Reagents and materials

3.1. Type II water, according to ISO standard 3696

3.2. Ethanol: purity ≥ 96 % (CAS no. 64-17-5)

3.3. Hydrogen: minimum specifications: 99.999% purity (CAS no. 1333-74-0)

3.4. Helium: minimum specifications: 99.999% purity (CAS no. 7440-59-7)

3.5. Methanol: purity ≥ 99 % (CAS no. 67-56-1)

3.6. 4-Methyl-2-pentanol (internal standard): purity ≥ 98 % (CAS no. 108-11-2). Internal standard used in the validation.

Note 1: Other internal standards can be used, such as:

- 3-pentanol: purity ≥ 98% (CAS no. 584-02-1)
- 4-methyl-1-pentanol: purity ≥ 98% (CAS no. 626-89-1)
- Methyl nonanoate: purity ≥ 98% (CAS no. 1731-84-6)

3.7. Reference materials: these may be, for example, wines from laboratory proficiency tests.

3.8. Preparation of working solutions (by way of example):

3.8.1. Approximately 10% v/v aqueous-alcoholic mixture

This mixture should be as close as possible to the alcohol content of the wine to be analysed. Pour 100 mL of ethanol (3.2) into a 1 L calibrated flask (4.2), make up to volume with demineralised water (3.1) and mix.

3.8.2. 10 g/L Internal standard solution

Using an analytical balance (4.1), weigh approximately 1 g of internal standard (3.6) into a 100 mL calibrated flask (4.3) that contains around 60 mL of 10% ethanol solution (3.8.1), so as to minimise evaporation of the internal standard. Make up to volume with the ethanol solution (3.8.1) and mix.

3.8.3. 1 g/L Internal standard solution

Add 10 mL of the 10 g/L internal standard solution (3.8.2) using a pipette (4.8) and make up to 100 mL (4.3) using the 10% v/v hydroalcoholic mixture (3.8.1).

3.8.4. 5 g/L Methanol stock solution

Using an analytical balance (4.1), weigh approximately 500 mg of methanol (3.5) into a 100 mL calibrated flask (4.3) that contains about 60 mL of 10% ethanol solution (3.8.1), so as to minimise evaporation of the methanol. Make up to volume with the ethanol solution (3.8.1) and mix.

3.8.5. Working calibration solutions

By way of example, a method for plotting a calibration curve is outlined below.

Each solution should be prepared with the 10% aqueous-alcoholic mixture (3.8.1).

3.8.5.1. 500 mg/L Methanol standard solution

Add 10 mL of the 5 g/L stock solution (3.8.4) to a 100 mL calibrated flask (4.3) using a pipette (4.8) and make up to volume with the 10% v/v ethanol solution (3.8.1).

3.8.5.1.1. 250 mg/L Methanol standard solution

Add 10 mL of the 500 mg/L methanol solution (3.8.5.1) to a 20 mL calibrated flask (4.5) using a pipette (4.8) and make up to volume with the 10% v/v ethanol solution (3.8.1).

3.8.5.1.2. 200 mg/L Methanol standard solution

Add 20 mL of the 500 mg/L methanol solution (3.8.5.1) to a 50 mL calibrated flask (4.4) using a pipette (4.7) and make up to volume with the 10% v/v ethanol solution (3.8.1).

3.8.5.1.3. 150 mg/L Methanol standard solution

Add 6 mL of the 500 mg/L methanol solution (3.8.5.1) to a 20 mL calibrated flask (4.5) using a pipette (4.9) and make up to volume with the 10% v/v ethanol solution (3.8.1).

3.8.5.1.4. 100 mg/L Methanol standard solution

Add 4 mL of the 500 mg/L methanol solution (3.8.5.1) to a 20 mL calibrated flask (4.5) using a pipette (4.10) and make up to volume with the 10% v/v ethanol solution (3.8.1).

3.8.5.1.5. 50 mg/L Methanol standard solution

Add 2 mL of the 500 mg/L methanol solution (3.8.5.1) to a 20 mL calibrated flask (4.5) using a pipette (4.11) and make up to volume with the 10% v/v ethanol solution (3.8.1).

4. Apparatus

4.1. Analytical balance (1 mg precision)

4.2. 1 L Class A calibrated flasks

4.3. 100 mL Class A calibrated flasks

4.4. 50 mL Class A calibrated flasks

4.5. 20 mL Class A calibrated flasks

4.6. 10 mL Class A calibrated flasks

4.7. 20 mL Class A pipettes with two marks

4.8. 10 mL Class A pipettes with two marks

4.9. 6 mL Class A pipettes with two marks

4.10. 4 mL Class A pipettes with two marks

4.11. 2 mL Class A pipettes with two marks

4.12. 1 mL Class A pipettes with two marks or 1 mL micropipettes

4.13. Temperature-programmable gas chromatograph with a flame ionisation detector and a data processing system capable of calculating areas or measuring peak heights

4.14. Fused silica capillary column coated with a Carbowax 20M-type polar stationary phase (for example):

- Chrompack CP-wax 57 CB, 50 m x 0.32 mm x 0.45 µm
- DB-WAX 52, 30 m x 25 mm x 0.2 µm

5. Sample preparation

Sparkling and/or young wines must be pre-degassed, for example, by mixing 200 mL of wine in a 1 L flask. Subsequently, the samples are distilled according to the method for determining alcoholic strength by volume (OIV-MA-AS312-01A). The distillation can be carried out without adding calcium hydroxide in this case.

5.1. Addition of internal standard (by way of example)

Pour 10 mL of distillate into a 10 mL calibrated flask (4.6), add 1 mL (4.12) of internal standard solution (3.8.3) and mix.

6. Procedure

The calibration curve standards are treated in the same way as the samples (point 5.1).

It is recommended that the aqueous-alcoholic mixture (3.8.1) is injected at the start of the sequence in order to verify that it does not contain methanol.

6.1. Operating conditions (as a guide):

Carrier gas: helium or hydrogen

Carrier gas flow: 7 mL/min

Injection: split (ratio: 7:50)

Injection volume: 1 or 2 µL

Injector temperature: 200-260 ºC

Detector temperature: 220-300 ºC

Temperature programme: from 35 ºC (for 2 minutes) to 170 ºC, at 7.5 ºC/min

7. Calculations

Calculate the concentration of methanol (C_i), using the following equation:

$$C_i\ [\text{mg/L AA}] = RRF_i^{ethanol} \cdot \frac{A_i}{A_{ethnol}} \cdot \rho_{ethanol}$$

$RRF_i^{ethanol}$ - relative response factor for methanol relative to ethanol

A_i – peak area of methanol

$A_{ethanol}$ – peak area of ethanol

$\rho_{ethanol}$ – concentration of ethanol

8. Expression of the results

The concentration of methanol is may be expressed in mg/L or in mg/100 mL absolute alcohol; in the latter case, the alcohol content by volume of the wine should be determined.

Note 2: mg/100 mL absolute alcohol = mg/L x 10/alcohol content by volume

9. Precision

The data from the international interlaboratory test is outlined in Annex A.

10. Quality control

Internal quality control may be carried out using certified reference materials or wines whose characteristics have been determined from a consensus (3.7). These should be prepared as for the samples (point 5). Participation in proficiency tests is recommended.

11. Report of the results

The results are expressed to the nearest whole number (in accordance with the uncertainty).

12. Bibliography

Compendium of international methods of wine and must analysis. Method OIV-MA-AS312-01A (Alcoholic strength).

Annex A
Statistical results of the interlaboratory test

Design of validation study

The validation study was conducted with 10 samples: 2 white wines, one dry and one sweet, 2 red wines, one of which was oaked, and 1 fortified wine (Port), including blind duplicates, according to OIV recommendations. The approximate concentration of methanol is shown in the following table.

Sample	White wine Dry	White wine Sweet	Red wine	Red wine oaked	Fortified wine port
Methanol (mg/L)	50	150*	270	400*	120

(*) In this particular indicated case, methanol was added to the wine to cover a greater range of concentrations. The wine was then mixed, stabilised and bottled.

Participating laboratories:

Samples were sent to 17 laboratories in 9 different countries.

Laboratorios Agroalimentarios, Madrid (Spain)

Estación de Viticultura y Enología de Galicia, EVEGA (Spain)

Estació de Viticultura i Enologia de Vilafranca del Penedès, (Spain)

Estación Enológica de Haro, La Rioja (Spain)

Estación de Viticultura y Enología de Galicia (Spain)

Lab. Bordeaux, Service Commun des Lab., Pessac (France)

Laboratoire d'Ile-de-France, Paris (France)

Laboratoires Inter Rhône (France)

Comité Interprof. du Vin de Champagne (CIVC) (France)

Bfr-Bundesinst. f. Risikobewertung (Germany)

Landesuntersuchungsamt Mainz (Germany)

Instituto Nacional de Vitivinicultura, Mendoza (Argentina)

ALKO Inc., Alcohol Control Lab. (ACL) (Finland)

Instituto dos Vinhos do Douro e do Porto (Portugal)

Czech Agriculture and Food Inspection Authority (CAFIA), Brno (Czech Republic) CZ

National Food Safety Office, Directorate of Oenology and Alcoholic Beverages (NÉBIH BAII), Budapest (Hungary)

Lehr- und Forschungszentrum, Klosterneuburg (Austria)

Collaborative study on methanol

	Dry white		Sweet white		Red		Oaked red		Port	
Laboratory code	A	G	B	H	C	I	D	J	E	K
A	39.99	38.13	127.42	136.25	144.80	145.71	496.53	513.00	192.13	219.39
B	41.20	40.90	157.60	160.50	150.40	146.90	484.90	477.80	222.40	219.60
C	36.80	35.60	133.50	129.20	119.10	134.10	454.10	478.40	197.00	174.80
D	36.00	39.60	**177.40**	**145.50**	160.80	138.00	**302.00**	**494.50**	216.10	248.50
E	**68.00**	**70.00**	163.00	169.00	178.00	177.00	503.00	495.50	225.00	227.00
F	37.00	37.10	148.30	148.20	143.40	142.40	484.10	474.00	206.30	206.90
G	41.40	42.30	152.60	152.40	149.70	150.50	489.60	491.10	216.60	217.20
H	36.80	32.40	140.80	129.10	128.00	137.70	440.60	429.30	187.50	192.80
I	42.90	43.30	153.50	155.50	139.70	147.40	468.30	456.10	225.30	225.60
J	40.90	40.60	155.50	154.60	148.50	149.10	496.40	499.80	217.10	217.00
K	39.30	36.20	**103.10**	**143.10**	131.90	115.90	**437.90**	**334.00**	156.10	172.60
L	35.00	39.00	164.00	167.00	157.00	160.00	492.00	508.00	249.00	220.00
M	43.60	43.40	157.30	154.90	155.50	158.90	506.80	496.10	217.70	219.50
N	34.20	33.60	126.50	125.70	125.90	133.60	429.10	429.00	192.10	188.90
O	34.00	35.70	149.00	154.80	144.20	141.80	482.80	473.60	210.40	218.10
P	44.70	43.70	151.60	146.90	140.70	147.60	451.20	472.80	205.40	205.80
Q	40.70	38.80	153.00	149.80	158.20	153.40	498.20	497.50	225.50	217.20

Note: The values in bold correspond to values rejected according to the Cochran (variance outliers) and Grubbs (mean outliers) tests.

Indicators	Dry white	Sweet white	Red	Oaked red	Port
Number of accepted laboratories	16	15	17	15	17
Number of repetitions	2	2	2	2	2
Minimum	32.40	125.70	115.90	429.00	156.10
Maximum	44.70	169.00	178.00	513.00	249.00
Repeatability variance s_r^2	2.2466	12.1330	39.0164	76.3567	105.3390
Intergroup variance s_r^2	9.61893	146.39249	151.90249	535.61827	292.14282
Reproducibility variance s_r^2	11.8655	158.5254	190.9189	611.9750	397.4819
Overall mean	38.90	148.92	145.76	478.97	210.37
Repeatability standard deviation	1.50	3.48	6.25	8.74	10.26
r Limit	4.242	9.858	17.677	24.729	29.046
Repeatability CV	3.9	2.3	4.3	1.8	4.9
Reproducibility standard deviation	3.44	12.59	13.82	24.74	19.94
R Limit	9.748	35.632	39.103	70.009	56.422
Reproducibility CV	8.9	8.5	9.5	5.2	9.5
Horwitz RSD	6.09	4.97	4.99	4.17	4.72
Horrat r	0.6	0.5	0.9	0.4	1.0
Horwitz RSD	9.22	7.53	7.56	6.32	7.15
Horrat R	1.0	**1.1**	**1.3**	0.8	**1.3**

According to the Horrat values, the repeatability and reproducibility of the method are acceptable

Z-scores obtained by the participants: of the 85 Z-scores, 3 are unsatisfactory and 4 are questionable

	Z-score	Z-score	Z-score	Z-score	Z-score
Laboratory code	Dry white wine	Sweet white wine	Red wine	Oaked red wine	Port
A	0.05	-1.36	-0.04	1.04	-0.23
B	0.62	0.80	0.21	0.10	0.53
C	-0.78	-1.40	-1.39	-0.51	-1.23
D	-0.32	1.00	0.26	**-3.26**	1.10
E	**8.74**	1.36	**2.30**	0.81	0.78
F	-0.54	-0.05	-0.21	0.00	-0.19
G	0.86	0.28	0.31	0.46	0.33
H	-1.25	-1.11	-0.93	-1.78	-1.01
I	1.22	0.44	-0.16	-0.68	0.76
J	0.54	0.49	0.22	0.77	0.34
K	-0.33	**-2.05**	-1.58	**-3.76**	**-2.31**
L	-0.55	1.32	0.92	0.85	1.21
M	1.34	0.57	0.83	0.91	0.41
N	-1.45	-1.81	-1.16	**-2.02**	-1.00
O	-1.18	0.24	-0.20	-0.03	0.19
P	1.54	0.03	-0.12	-0.69	-0.24
Q	0.25	0.20	0.73	0.76	0.55