OIV-MA-AS315-28 Method of determination of 1,2-propanediol and 2,3-butanediol

Type IV method

1. Introduction

Measurable quantities of 1,2-propanediol and 2,3-butanediol are formed following fermentation processes. These compounds are practically absent in unfermented musts, yet found within certain limits in wines.

2. Principle

The analytes and the internal standard are extracted through the use of ethyl ether. Their transfer into the organic phase is facilitated by the increase in the ionic strength of the initial wine or must matrix. A large quantity of K_2CO_3 is added to the samples ('salting out') for this purpose. The extracts are analysed directly via GC-MS on a polar column. The detection is conducted according to the retention time and the mass spectrometer.

3. Scope of application

The method is suitable for determining 1,2-propanediol and 2,3-butanediol in musts and wines whose sugar content is greater than 20 g/L and whose analyte concentrations are between 1 mg/L and 500 mg/L.

4. Abreviations

C	Concentration
PG	1,2-Propanediol
GC-MS	Gas Chromatograph-Mass Spectrometer
H_2	Hydrogen
IS	Internal standard 1,3-butanediol
m/z	Mass/charge ratio
RF	Response factor

ML	Matrix calibration level
SS CS	Stock solution Calibration solution
SS CS	Stock solution Calibration solution
RT	Retention time
CS	Calibration solutions for gas chromatography
BG	2,3-Butanediol
S	Wine with a sugar content > 20 g/L
M	Must

5. Reagents

5.1. K_2CO_3 (CAS no. 584-08-7)

5.2. Ethyl ether (CAS no. 60-29-7)

5.3. Absolute ethanol (CAS no. 64-17-5)

5.4. Fructose (CAS no. 57-48-7)

5.5. Glucose (CAS no. 50-99-7)

5.6. Glycerol (CAS no. 56-81-5)

5.7. 1,2-Propanediol, purity > 99% (CAS no. 57-55-6)

5.8. 2,3-Butanediol, purity > 99%, mix of (R,R)- and (R,S)-isomers (CAS no. 513-85-9). Estimate the relative quantity of the (R,R) and (R,S) forms as follows:

5.8.1. prepare a 100 mg/L solution following the instructions in points 7.2.1 and 7.3, diluting the mix of 2,3-butanediol isomers (5.8) in water (5.10) instead of in the matrix model solution;

5.8.2. inject into the GC, under the conditions described in point 7.6, and calculate the percentage of (R,R) and (R,S) forms from the percentage of the areas of the two peaks;

5.8.3. take into consideration the relative quantity of the two forms to calculate the concentration of the calibration solutions, $C_{CS,i,}$, used in paragraph 8.2.1 for the

calculation of the RF_i relating to the (R,R) and (R,S) forms.

5.9. 1,3-Butanediol, purity > 99%, anhydrous (or dehydrated with sodium sulphate for 24 hours) (CAS no. 107-88-0)

5.10. Purified water for laboratory use, certified to the EN ISO 3696 standardNitrogen

6. Apparatus

6.1. Everyday laboratory apparatus such as class-A 1000-mL, 200-mL and 100-mL flasks

6.2. Analytical scale with an accuracy of ± 0,0001 g

6.3. Laboratory centrifuge (at least 4000 rpm or 2000 xg)

Note 1. The unit 'xg' refers to the acceleration experienced by particles in a centrifuge, while 'rpm' represents the number of revolutions the rotor of the centrifuge makes per minute. There is a relationship between these units of measurement:

$xg = 1.1178 \cdot 10^{-3} \cdot n^2 \cdot r$. *In the laboratory that developed this method, r = 0.115 m.*

6.4. Chromatograph coupled to a mass spectrometer and split-splitless injector

6.5. Precision micropipettes and Pasteur pipettes

6.6. 30-mL centrifuge tubes resistant to ether and provided with stoppers

6.7. Thermostatically-controlled water bath

6.8. Vertical vortex mixer

7. Procedure

7.1. Preparation of the model solutions that simulate the matrix

To obtain a better response to the GC-MS during quantification, different solutions should be prepared that simulate the matrix of the sample in question as much as possible, given that the response to the analysis of glycols varies according to the matrix in which they were diluted.

Table 1: Preparation of the model solutions in 1000-mL calibrated flasks.

	Model solution	
	M	S
Fructose	100 g/L	50 g/L
Glucose	100 g/L	50 g/L

Glycerol	1 g/L	4 g/L
Absolute ethanol	1% v/v	5% v/v

7.2. Preparation of reference solutions

7.2.1. SS: PG and BG stock solutions

With an accuracy of 0.1 mg, weigh about 0.10 g of 1,2-propanediol (PG) and about 0.10 g of 2,3-butanediol (BG) into a 10-mL calibrated flask and fill up to the calibration mark with water (5.10). Make a note of the weights. Hermetically seal the flask and mix. The concentration is approximatively 10 mg/mL in the PG solution and 10 mg/mL in the BG solution.

If the quantities of PG and BG differ by 0.1 g, calculate the exact concentrations based on the weights noted.

7.2.2. IS: IS stock solution

With an accuracy of 0.1 mg, weigh about 0.10 g of 1,3-Butanediol (IS) into a 10-mL calibrated flask and fill up to the calibration mark with water (5.10). Make a note of the weight. Hermetically seal the flask and mix. The concentration of this solution is 10 mg/mL.

If the quantity of IS differs by 0.1 g, calculate the exact concentration based on the weights noted.

7.3. Preparation of the calibration matrix solution

The calibration solutions are prepared as follows, by diluting the SS solution into a model solution whose composition is as close as possible to that of the sample for analysis (for sweet wine, S model solution; for must, M model solution):

Table.2: Preparation of calibration solutions (CS) in 100-mL calibrated flasks:

	CS-M	CS-S
SS Solution	1 mL	1 mL
To reach a final volume of 100 mL, make up to volume with:	M model solution	S model solution

Every CS calibration solution contains the selected matrix and the concentration of PG and BG is 100 mg/L. The internal standard is added before the extraction as described in paragraph 7.5.

7.4. Preparation of samples

If the analyte concentration in the sample is greater than the maximum concentration provided within the scope of application, dilute the sample with the model solution (7.1).

Stir the sample before taking the 10 mL to be extracted.

In the case of musts or cloudy wines, sample the clear wine after filtration.

In the case of sparkling or semi-sparkling wines, carry out degassing as described in the OIV method 'Total Acidity' (OIV-MA-AS313-01, point 5.1). Proceed with all of the preparation and carry out the tests in duplicate.

7.5. Extraction

7.5.1. Adding the internal standard (IS) to the sample

Prepare a solution containing 5 mL of IS solution (7.2.2) in a 100-mL flask and fill up to the calibration mark with the sample to be analysed, then shake well.

This solution contains 500 mg/L of IS.

7.5.2. Musts and wines with a sugar content > 20 g/L

7.5.2.1. Addition of K_2CO_3

Pour 10 mL of the solution just prepared, composed of the sample to be analysed and the IS solution, into the centrifuge test tube (6.6), then add 10 g of K_2CO_3 (5.1) and wait for it to cool. To speed up cooling you may use a thermostatically-controlled water bath at 20 °C (6.7).

7.5.2.2. Extraction with ether

Once cooled, add 10 mL of ethyl ether (5.2) and shake the whole mixture with a vertical vortex agitator, then put it in the centrifuge (6.3) at about 3500 rpm (or 1500 xg) for 10 minutes.

7.5.3. *Purification for GC/MS analysis*

The supernatant liquid is collected with a Pasteur pipette, transferred into a suitable flask and the solvent evaporated under a flow of nitrogen. The residue is recovered with about 1 mL of ethyl ether and placed in a tightly sealed GC vial ready for GC/MS analysis.

7.5.4. Extraction of the CS calibration solutions

This procedure must also be carried out for the chosen CS calibration solution (7.3). The CS solutions must be considered as samples to all intents and purposes, and must thus be treated in the same way as the sample starting from the moment the IS is added (7.5.1).

7.6. GC-MS analysis

By way of example, the specific parameters of the GC-MS analysis are given below.

Alternative systems may be used, if they give adequate chromatographic performances and make it possible to separate the chromatographic peaks with a precision of greater than 2.

7.6.1. GC typical conditions

Column: 60 m x 0.25 mm x 0.25 µm DB-WAX

Carrier gas: He

Carrier gas flow: 1.0 mL/min

Injector temperature: 250 °C

Injection volume: 1 µL

Ionising current: 70 eV

Temperature settings:

	Increase (°C/min)	Temperature (°C)	Time (min)
Start		50	8.00
Ramp 1	4.0	220	
Ramp 2		220	40

Specific MS conditions

Source: 230 °C

MS detector: 150 °C scan, 35.00 – 350.00 amu.

Start time: 10 min

Acquisition time for each mass is 250 µs

Acquisition mode: Full Scan

8. Evaluation

8.1. Identification

Identification is performed by comparing the retention time of the calibration solutions provided for this purpose and the mass spectrum found in the library associated with the GC-MS.

8.2. Calculations

For quantification, m/z = 45 is used for the IS, and also the PG and two forms of BG.

8.2.1. *Determination of response factors*

Quantification is carried out based on the response factor RF obtained by analysing

the reference solution:

$$RF_i = \frac{A_{IS}/C_{IS}}{A_{CS,i}/C_{cs,i}}$$

where:

A_{IS} is the peak area of the internal standard and C_{IS} is its concentration;

$A_{CS,i}$ is the peak area of the PG or each of the isomeric forms of BG in the calibration solution and $C_{cs,i}$ is its concentration.

8.2.2. *Calculation of the concentrations in the samples*

Once the response factor RF has been calculated the calculation of the concentration of PG and each of the isomeric forms of BG in the samples can be performed, according to the following formula:

$$C_i = RF_i \times C_{CS} \times A_i/A_{is}$$

where:

A_i is the peak area of the PG or the BG in the sample and C_i is its concentration.

8.3. Expression of the results

The results are expressed in mg/L to the nearest whole number.

Express 2,3-butanediol as the sum of (R,R)-2,3-butanediol and (R,S)-2,3-butanediol.

9. Bibliography

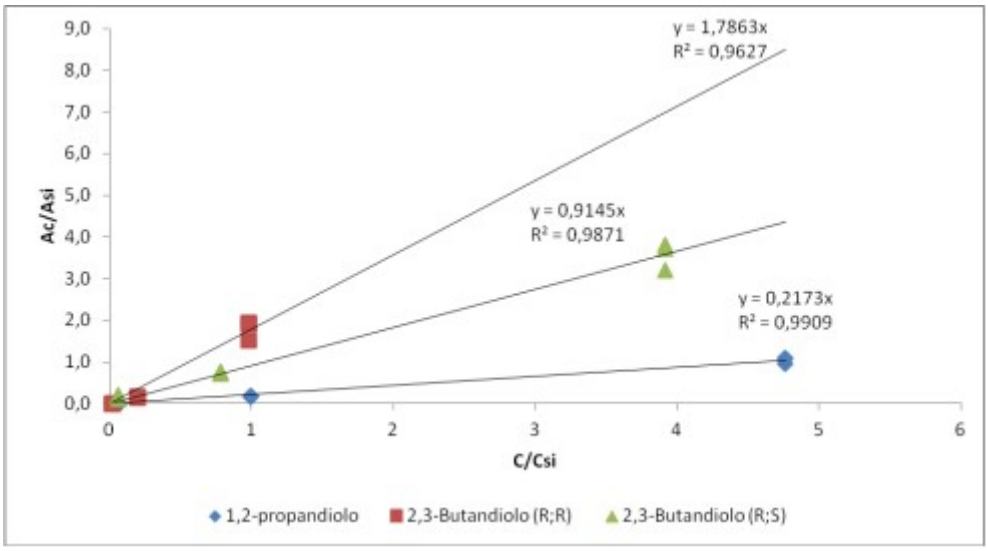
- Carsten Fauhl, Reiner Wittkowski, Janice Lofthouse, Simon Hird, Paul Brereton, Giuseppe Versini, Michele Lees and Claude Guillou, 'Gas Chromatographic/Mass Spectrometric Determination of 3-Methoxy-1,2-Propanediol and Cyclic Diglycerols, By-Products of Technical Glycerol, in Wine: Interlaboratory Study', *JOURNAL OF AOAC INTERNATIONAL*, VOL. 87, NO. 5, 2004.
- Di Stefano, R., García Moruno, E. and Borsa, D., 'Proposta di un metodo di preparazione del campione per la determinazione dei glicoli dei vini', VINI D'ITALIA, 4, 1992, pp. 61-64.
- García Moruno, E. and Di Stefano, R., 'La determinazione del glicerolo, del 2,3-butandiolo e dei glicoli nei vini', VINI D'ITALIA, 5, 1989, pp. 41-46.
- Di Stefano, R., Borsa, D. and García Moruno, E., 'Glicoli naturalmente presenti

nei vini', VINI D'ITALIA, 5, 1988, pp. 39-44.

Annex 1 :Method performance

1. Linearity

Verification of the linearity of the response for a 200 g/L sugar solution (100 g/L of glucose and 100 g/L of fructose). Each analyte was added at concentrations of 10, 100 and 500 mg/L, while the IS was added at a concentration of around 100 mg/L. The measurements were repeated three times.



The mean response factors are

1,2-Propanediol RF =$(C/C_{IS})/(A/A_{IS})$ = 1/0.2173 = 4.60

(R,R)-2,3-Butanediol RF = $(C/C_{IS})/(A/A_{IS})$ = 1/1.7863 = 0.56

(R,S)-2,3-Butanediol RF = $(C/C_{IS})/(A/A_{IS})$ = 1/0.9145 = 1.09

2. Repeatability

The repeatability was evaluated for two must samples.

One was analysed as such (Must N°1) and the other was obtained by adding 100 mg/L of SS stock solution to it (Must N°2).

The following table makes reference to 10 repeated analyses, and the repeatability (r) is calculated according to the formula r = 2.8*Sr. (Sr = repeatability standard deviation, RSDr = relative standard deviation of repeatability).

Compound	Must N°1	Must N°2

	Mean (mg/L)	Sr (mg/L)	RSDr (%)	r (mg/L)	Mean (mg/L)	Sr (mg/L)	RSDr (%)	r (mg/L)
1,2-Propanediol	1.5	0.5	36	1.5	107	9	9	30
(R,R)-2,3-Butanediol	3.2	1.6	52	4.6	30	3	9	9.0
(R,S)-2,3-Butanediol	5.4	1.7	33	4.9	104	11	10	34

Evaluation of the precision of the limit of repeatability according to the Horwitz equation and Horrat parameter (r):

Must no. 1

	Mean (mg/L)	C□10^6 (m/m)	PRSD (R)	R Horwitz	Horrat (r)	r min H	r max H
1,2-Propanediol	1.5	1.5	15	0.6	2.40	0.2	0.8
(R,R)-2,3-Butanediol	3.2	3.2	13	1.2	3.87	0.3	1.6
(R,S)-2,3-Butanediol	5.4	5.4	12	1.9	2.64	0.5	2.5

The limit of repeatability ‘r’ is not contained within the validation range specified by the Horwitz equation (r min H < r < r max H) due to the greater volatility of low-concentration measurements, close to the limit of quantification established in paragraph 5 of Annex 1.

Must no.2

	Mean (mg/L)	C□10^5 (m/m)	PRSD (R)	R Horwitz	Horrat (r)	r min H	r max H
1,2-Propanediol	107	11	7.9	24	1.09	5.9	31.8
(R,R)-2,3-Butanediol	30	3	9.5	8	0.98	2.0	10.7
(R,S)-2,3-Butanediol	104	10	7.9	23	1.29	5.7	30.8

The limit of repeatability ‘r’ is not contained within the validation range specified by the Horwitz equation (r min H < r < r max H).

3. Recovery rate

The recovery rate was evaluated for must N°2 before and after addition of the SS stock solution, as described in paragraph 7.3 of the method.

Compound	C. in the must (mg/L)	C. added (mg/L)	Theoretical C. (mg/L)	Measured C. (mg/L)	Recovery rate (%)
1,2-Propanediol	0.7	99.5	100.2	107.5	107
(R,R)-2,3-Butanediol	12.6	21.7	34.3	29.9	87
(R,S)-2,3-Butanediol	11.4	86.8	98.2	103.7	106
(R,R)- + (R,S)-2,3-Butanediol	*24.0*	*108.5*	*132.5*	*133.6*	*101*

The recovery rate is satisfactory for 1,2-propanediol and for 2,3-butanediol evaluated overall as the sum of both forms.

4. Effect of the sugar matrix on the response factors

The RFs obtained for the equimolar glucose and fructose solutions with total sugar concentrations of 200 g/L and 2 g/L were compared.

	1,2-Propanediol		(R,R)-2,3-Butanediol		(R,S)-2,3-Butanediol	
Sugars	200 g/L	2 g/L	200 g/L	2 g/L	200 g/L	2 g/L
RF	4.60	5.90	0.55	0.56	1.08	1.09
$\lvert\Delta RF\rvert\%$	22.0 %		1.8 %		0.9 %	

The effect of the matrix on 1,2-Propanediol is highly marked, while it is negligible for both forms of 2,3-Butanediol.

5. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) depend on specific analytical-chemical measurement conditions and should be determined by all those who use the method.

The limit of detection (LOD) and limit of quantification (LOQ) were evaluated using the

above-mentioned equipment and conditions (point 8) and by following the instructions of Resolution OENO 7-2000 (OIV-MA-AS1-10) 'Estimation of the detection and quantification limits of a method of analysis' as described in paragraph 4.2 concerning the "Graph" Approach.

	1,2-Propanediol	(R,R)-2,3-Butanediol	(R,S)-2,3-Butanediol
LOD (mg/L)	0.2	0.2	0.2
LOQ (mg/L)	0.6	0.7	0.8

Annex 2

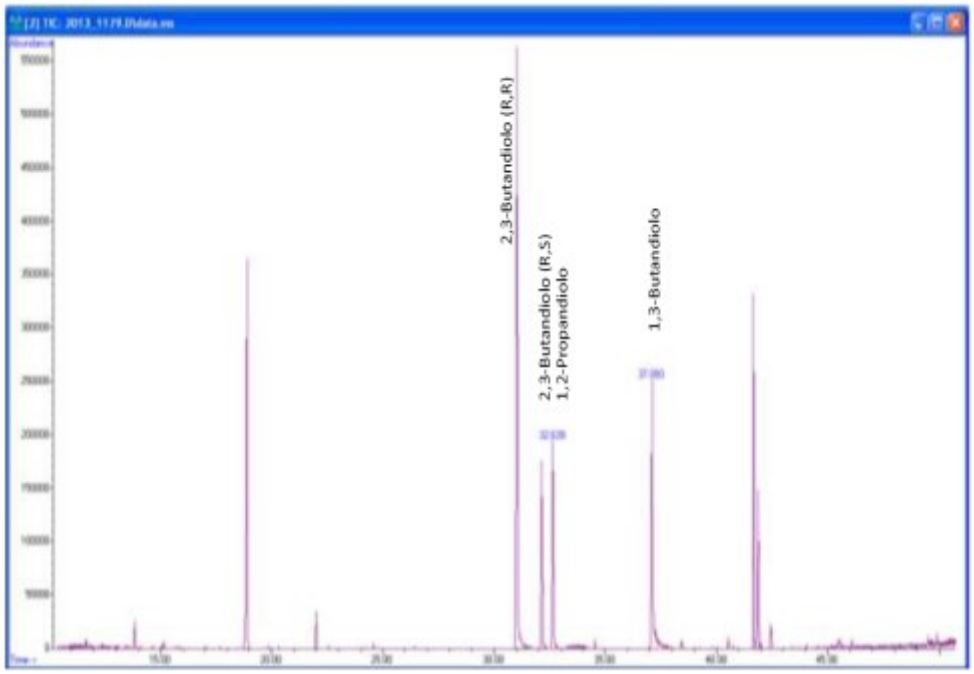


FIG. 1 Chromatogram of a wine