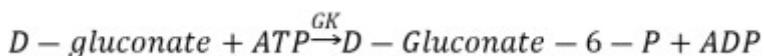


**OIV-MA-AS313-28 Determination of D-gluconic acid in wines and musts by automated enzymatic method****Type II method****1. Scope of application**

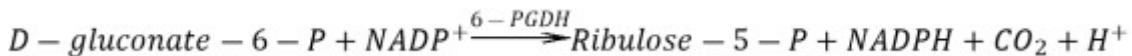
This method makes it possible to determine D-gluconic acid in wines and musts by specific enzymatic analysis using an automatic sequential analyser, with concentrations of 0.06 g/L to 5.28 g/L of analyte (taking into account that the sample may be diluted).

**2. Principle**

The D-gluconate present in the sample is phosphorylated by adenosine triphosphate (ATP) during an enzymatic reaction catalysed by gluconate kinase (GK), to produce D-gluconate 6-phosphate and adenosine diphosphate (ADP).



In the presence of nicotinamide adenine dinucleotide phosphate (NADP), D-gluconate 6-phosphate oxidises to form ribulose 5-phosphate through the action of enzyme 6-phosphogluconate dehydrogenase (6-PGDH). The quantity produced of reduced nicotinamide adenine dinucleotide phosphate (NADPH) corresponds to that of D-gluconate-6-phosphate and, as such, of D-gluconic acid.



Reduced nicotinamide adenine dinucleotide phosphate (NADPH) is determined by spectrophotometry at 340 nm (the wavelength of maximum absorption of NADPH). The NADPH concentration is proportional to the concentration of D-gluconic acid.

**3. Reagents and working solutions****3.1. Reagents:**

- 3.1.1. Distilled water for laboratory use, certified to the EN ISO 3696 standard
- 3.1.2. PIPES (Piperazine-1,4-bis[ethanesulfonic acid]) (CAS No. 5625-37-6)
- 3.1.3.  $\square\text{-NADP-Na}_2$  ( $\square$ -Nicotinamide adenine dinucleotide phosphate, disodium salt) (CAS No. 24292-60-2)
- 3.1.4.  $MgCl_2\cdot 6H_2O$  (Magnesium chloride hexahydrate) (CAS No. 7791-18-6)
- 3.1.5. ATP- $Na_2$  (Adenosine 5'-triphosphate disodium salt) (CAS No. 987-65-5)

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

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3.1.6. Gluconate kinase (GK) (EC 2.7.1.12)

3.1.7. 6-phosphogluconate dehydrogenase (6-PGDH) (EC 1.1.1.44)

3.1.8. D-gluconic acid sodium salt (CAS No.527-07-1), minimum purity  $\geq$  99%

3.1.9. NaOH (Sodium hydroxide) (CAS No. 1310-73-2)

3.1.10. PVP K-90 (Polyvinylpyrrolidone K-90) (CAS No. 9003-39-8)

### 3.2. Working solutions

3.2.1. Reagent 1: dissolve 30.2g PIPES (3.1.2) (100 mmol/L), 1 g  $\square$ -NADP-Na<sub>2</sub> (3.1.3) (1.3 mmol/L), 5.28 g NaOH (3.1.9) and 5 g PVP K-90 (3.1.10) in 1 L distilled water (3.1.1). The pH should be in the 6.3-6.4 range. This solution is stable for at least 4 weeks at 2-8 °C.

3.2.2. Reagent 2: dissolve 30.2 g PIPES (3.1.2) (100 mmol/L), 1 g MgCl<sub>2</sub>·6H<sub>2</sub>O (3.1.4) (1.3 mmol/L), 4.84 g ATP-Na<sub>2</sub> (3.1.5) and 7.6 g NaOH (3.1.9) in 1 L distilled water (3.1.1). The pH should be in the 7.0-7.2 range. Add 10 KU kinase glutonate (3.1.6) and 10 KU 6-phosphogluconate dehydrogenase (3.1.7). This solution is stable for at least 4 weeks at 2-8 °C.

### 3.3. Calibration solutions

Calibration solutions are prepared from the D-gluconic acid sodium salt (3.1.8), by weighing, in concentrations that cover the linear range of the method (0.06-2 g/L).

Note 1: The formulations described above are for preparing 1 L of reagent. Other volumes may be prepared according to the needs of the laboratory.

Note 2: Commercial kits are available for the determination of D-gluconic acid. The user should check that the kit includes the reagents mentioned above.

## 4. Apparatus

1. Sequential automatic analyser with temperature control (approximately 37 °C), adjusted to measure absorbance at 340 nm. The apparatus should have software that facilitates data acquisition and carries out the necessary calculations.
2. Spectrophotometer or photometer to measure absorbance at 340 nm
3. Glass, quartz or methacrylate cuvettes
4. Class-A glassware for regular laboratory use (flasks, pipettes, etc.)
5. Micropipettes
6. Analytical balance with a resolution of  $\pm$  0.0001 g
7. pH meter

## 5. Sample preparation

If necessary, follow the procedure for preparation of the corresponding sample:

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

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- 5.1. Filter or centrifuge the samples if they contain suspended particles.
- 5.2. Degas samples that contain carbon dioxide through stirring under vacuum, an ultrasonic bath or any other means that makes it possible to reach the required level of degasification.
- 5.3. Samples with a concentration higher than the specified limit of linearity (2 g/L) should be diluted with distilled water (3.1.1). Multiply the concentration obtained by the dilution factor.

### 6. Procedure

Given that different types of analysers may be used, it is recommended to strictly follow the manufacturer's instructions. This is also applicable to commercial enzymatic kits.

The procedures are those detailed below (volumes are given by way of example).

#### 6.1. Manual procedure

6.1.1. Preheat the reagents and photometer to 37 °C.

6.1.2. Add the following to a cuvette using a pipette:

	Reagent blank (RB)	Standard / Sample
Standard / Sample	-	33 µL
Distilled water	33 µL	-
Reagent 1	800 µL	800 µL

6.1.3. Mix and incubate for 1 min at 37 °C. Read the absorbance (A1) at 340 nm.

6.1.4. Add the following to the cuvette using a pipette:

Reagent 2	200 µL	200 µL
-----------	--------	--------

6.1.5. Mix and incubate for 10 min at 37 °C. Read the absorbance (A2) of the reagent blank, standard and sample at 340 nm.

#### 6.2. Automated procedure

6.2.1. Introduce the following parameters into the automatic analyser (which complies with the requirements in paragraph 4.1):

Wavelength: 340 nm

Temperature: 37 °C

Analysis mode: 2 points (differential)

Sample volume: 10 µL

Volume of Reagent 1: 240 µL

Volume of Reagent 2: 60 µL

6.2.2. Programme an application in the analyser so that it performs the following

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

sequence:

	Reagent blank (RB)	Standard / Sample
Standard / Sample	-	10 µL
Distilled water	10 µL	-
Reagent 1	240 µL	240 µL

Mix, incubate for 1-5 min and read the absorbance (A1). Then add:

Reagent 2	60 µL	60 µL
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Mix, incubate for 10 min and read the absorbance (A2).

The apparatus takes regular measurements, which makes it possible to obtain reaction kinetics (Fig. 1).

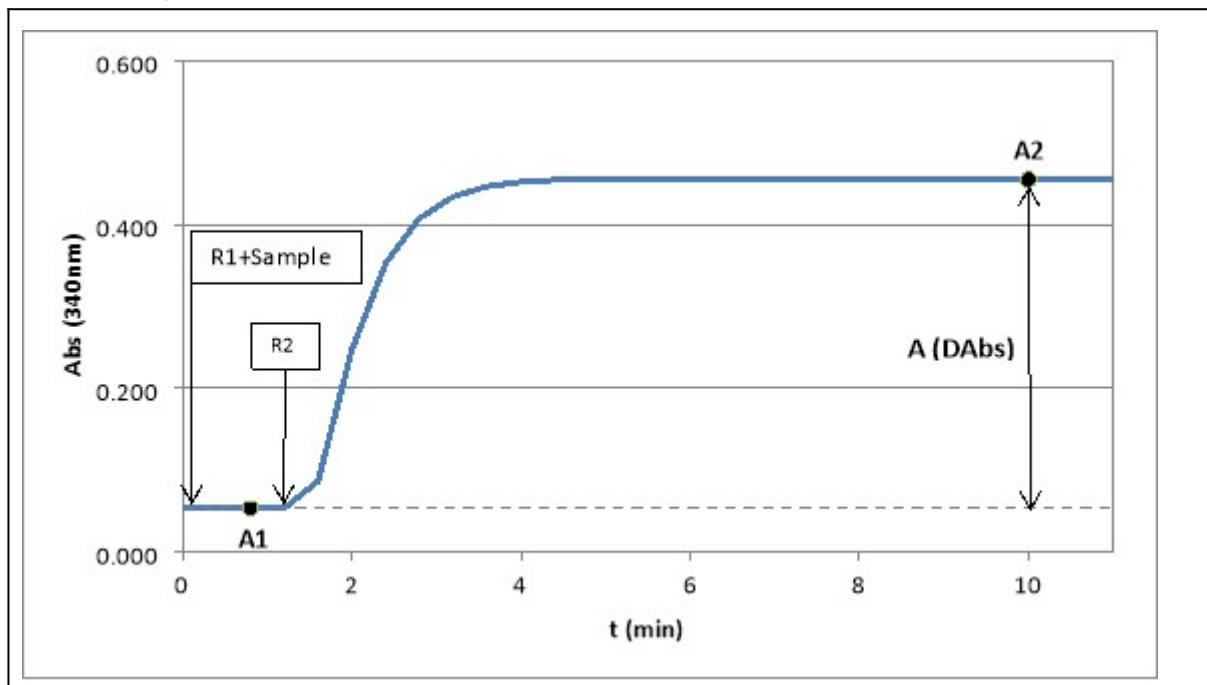


Figure 1: Example of reaction kinetics

6.2.3. It is advisable to check the calibration by carrying out three controls spread out over the measurement range. Each laboratory should establish its own internal quality-control programme, as well as correction procedures in case the controls do not comply with the acceptable tolerance levels.

## 7. Calculations

Calculate the D-gluconic acid concentration using the following formula:

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

$$\frac{(A2 - 0.81 \times A1)_{Sample} - (A2 - 0.81 \times A1)_{RB}}{A1_{RB}}$$

$$\frac{(A2 - 0.81 \times A1)_{Sample} - (A2 - 0.81 \times A1)_{RB}}{A1_{RB}}$$

$$\frac{(A2 - 0.81 \times A1)_{Sample} - (A2 - 0.81 \times A1)_{RB}}{A1_{RB}}$$

If the calibration is carried out with one point (standard)

and the blank:

$$\frac{(A2 - 0.81 \times A1)_{Sample} - (A2 - 0.81 \times A1)_{RB}}{(A2 - 0.81 \times A1)_{Standard} - (A2 - 0.81 \times A1)_{RE}} \times F \times g/L_{standard} = g/L_{sample}$$

$$\frac{(A2 - 0.81 \times A1)_{Sample} - (A2 - 0.81 \times A1)_{RB}}{A1_{RB}}$$

$$\frac{(A2 - 0.81 \times A1)_{Sample} - (A2 - 0.81 \times A1)_{RB}}{A1_{RB}}$$

$$\frac{(A2 - 0.81 \times A1)_{Sample} - (A2 - 0.81 \times A1)_{RB}}{A1_{RB}}$$

If the calibration is with a calibration line:

$$A = (A2 - 0.81 \times A1)_{Sample} - (A2 - 0.81 \times A1)_{RB}$$

The absorbance calculated (A) is interpolated on the calibration line (Fig. 2) to obtain the D-gluconic acid concentration. Multiply the concentration obtained by the dilution factor (F).

A1: absorbance of the Blank/Standard/Sample + Reagent 1

A2: absorbance of the Blank/Standard/Sample + Reagent 1 + Reagent 2

RB: reagent blank

0.81: factor of correction of the dilution of Reagent 1 (this may vary depending on the volumes used according to the formula [Sample vol. + Reagent 1] / [Sample vol. + Reagent 1 + Reagent 2]).

F: factor of dilution of the sample (to be applied if necessary)

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

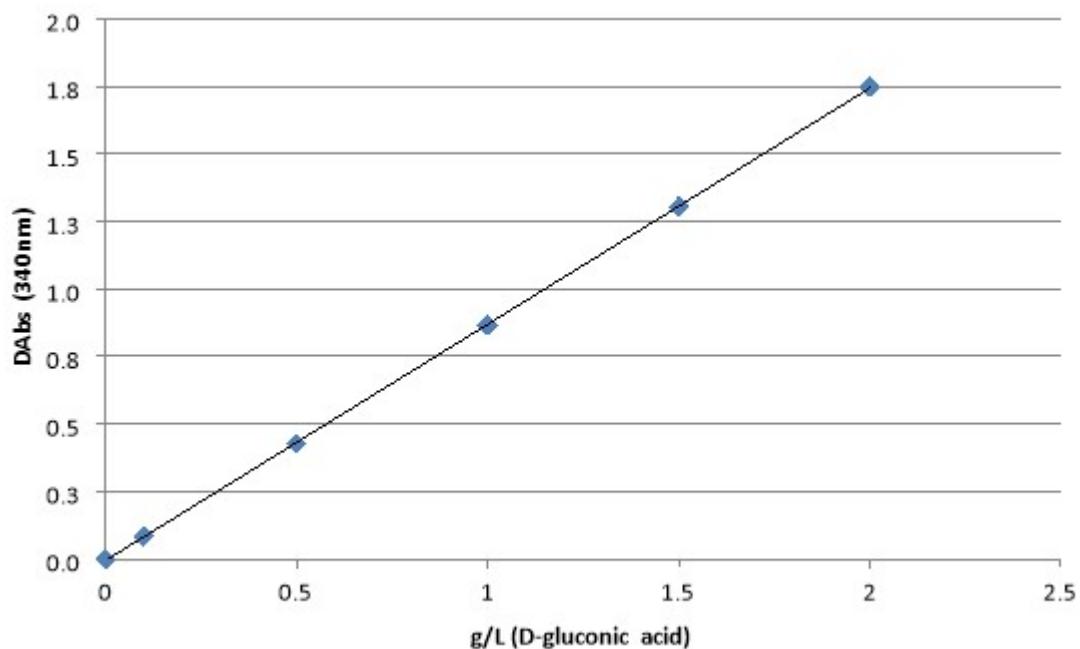


Figure 2: Example of a calibration line

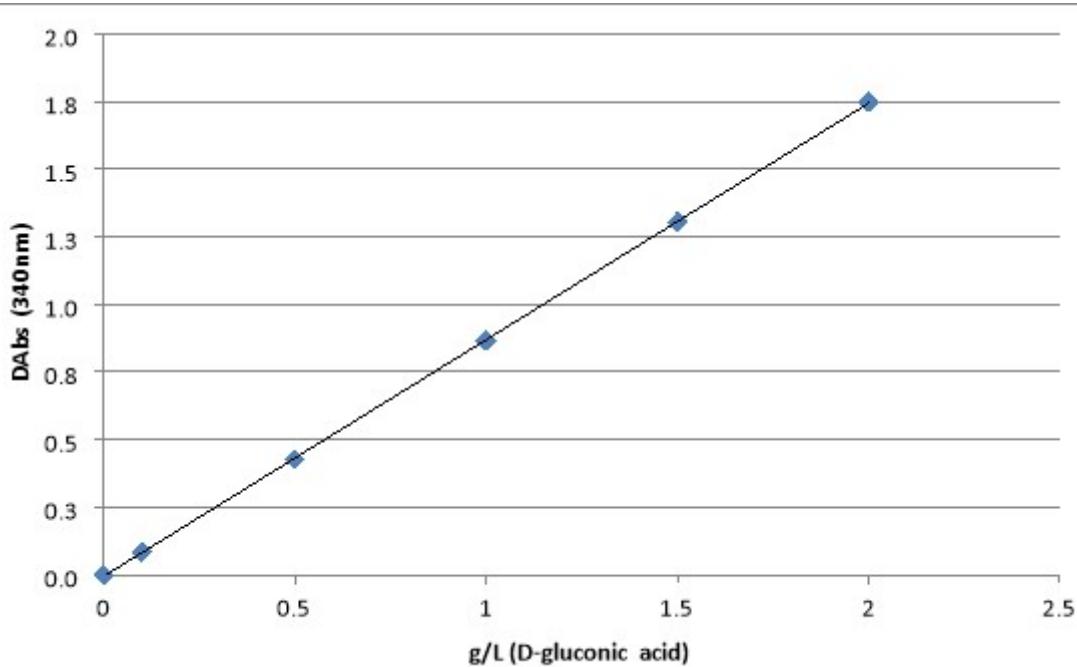


Figure 2: Example of a calibration line

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

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### 8. Expression of results

The results are expressed in g/L to 2 decimal points, or in accordance with the uncertainty.

### 9. Automated enzymatic method characteristics

#### 9.1. Repeatability

$$r = 0.0396x + 0.0098$$

With x representing the concentration of gluconic acid in g/L.

#### 9.2. Reproducibility

$$R = 0.1226x + 0.0237$$

With x representing the concentration of gluconic acid in g/L.

#### 9.3. Limit of quantification

Validated LoQ = 0.06 g/L

Annex =Results of the inter-laboratory study

### 1. Collaborative study

#### 1.1. Participating laboratories: 19 laboratories participated from 6 different countries.

Laboratory	Country
Agroscope	Switzerland
Biosystems S.A	Spain
Bundesamt für Weinbau	Austria
Bundesinstitut für Risikobewertung (BfR)	Germany
Centrolab 2006, S.L	Spain
Comité Champagne Comité Interprofessionnel du vin de Champagne (CIVC)	France
Estación de Viticultura y Enología de Navarra (EVENA)	Spain
Estación de Viticultura y Enología Alcázar de San Juan	Spain
Estación Enológica de Castilla y León (ITACyL)	Spain

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

Estación Enológica de Haro	Spain
Federal College and Research Institute for Viticulture and Pomology (HBLA)	Austria
Freixenet S.A	Spain
Institut Català de la Vinya i el Vi (INCAVI)	Spain
Instituto dos Vinhos do Douro e do Porto (IVDP)	Portugal
Laboratoires Dioenos Rhône	France
Laboratoires Dubernet	France
Laboratorio Arbitral Agroalimentario	Spain
Landesuntersuchungsamt, Institut für Lebensmittelchemie und Arzneimittelprüfung	Germany
Miguel Torres, SA	Spain

For analysis, use 2 x 10 blind duplicate samples, with 1 repetition.

### 1.2. Samples

Sample	Vial	Type of sample
A	1 / 12	Moscatel
B	2 / 11	Concentrated must
C	3 / 13	Sulphited must
D	4 / 15	White wine
E	5 / 14	White wine
F	6 / 16	Rosé wine
G	7 / 10	Red wine

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

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H	8 / 19	Red wine
I	9 / 18	Red wine
J	17 / 20	Synthetic matrix

### 1.3. Automated method results

		A		B		C		D		E		F		G		H		I		J	
		1	12	2	11	3	13	4	15	5	14	6	16	7	10	8	19	9	18	17	20
1	Rep #1	2.10	2.00	1.00	1.00	0.23	0.23	0.29	0.28	0.10	0.11	2.72	2.72	5.20	5.25	0.15	0.15	0.49	0.48	0.06	0.06
	Rep #2	2.01	2.02	0.99	1.00	0.22	0.23	0.30	0.29	0.11	0.10	2.70	2.68	5.13	5.18	0.14	0.14	0.48	0.49	0.05	0.06
	□ (1)	2.06	2.01	1.00	1.00	0.23	0.23	0.30	0.29	0.11	0.11	2.71	2.70	5.17	5.22	0.15	0.15	0.49	0.49	0.06	0.06
2	Rep #1	1.95	2.02	1.02	0.99	0.23	0.23	0.29	0.30	0.10	0.09	2.79	2.72	5.27	5.24	0.13	0.13	0.47	0.46	0.05	0.05
	Rep #2	2.00	2.10	1.03	1.01	0.23	0.23	0.29	0.30	0.11	0.10	2.75	2.80	5.30	5.20	0.13	0.13	0.47	0.45	0.05	0.05
	□ (2)	1.97	2.06	1.03	1.00	0.23	0.23	0.29	0.30	0.11	0.10	2.77	2.76	5.28	5.22	0.13	0.13	0.47	0.45	0.05	0.05
3	Rep #1	2.19	2.19	1.06	1.07	0.27	0.28	0.34	0.33	0.13	0.13	2.95	3.06	5.54	5.63	0.08	0.08	0.51	0.50	0.06	0.06
	Rep #2	2.21	2.30	1.09	1.07	0.27	0.28	0.34	0.34	0.13	0.13	2.95	2.99	5.51	5.68	0.07	0.08	0.49	0.50	0.06	0.06
	□ (3)	2.20	2.24	1.07	1.07	0.27	0.28	0.34	0.34	0.13	0.13	2.95	3.02	5.53	5.66	0.07	0.08	0.50	0.50	0.06	0.06
4	Rep #1	2.10	2.10	1.02	1.05	0.23	0.24	0.28	0.29	0.10	0.10	2.70	2.80	3.78	3.94	0.13	0.12	0.45	0.46	0.10	0.10
	Rep #2	2.08	2.12	1.03	1.04	0.24	0.25	0.29	0.29	0.10	0.11	2.72	2.82	3.80	3.98	0.13	0.13	0.45	0.45	0.10	0.10
	□ (4)	2.09	2.11	1.03	1.05	0.24	0.25	0.29	0.29	0.10	0.11	2.71	2.81	3.79	3.96	0.13	0.13	0.45	0.46	0.10	0.10
5	Rep #1	1.88	1.94	0.98	0.96	0.22	0.33	0.27	0.26	0.10	0.09	2.68	2.58	4.95	4.90	0.11	0.08	0.43	0.42	0.04	0.05
	Rep #2	2.06	2.12	0.99	1.00	0.24	0.23	0.29	0.29	0.11	0.09	3.08	2.84	5.40	5.30	0.12	0.15	0.47	0.48	0.07	0.06
	□ (5)	1.97	2.03	0.99	0.98	0.23	0.28	0.28	0.28	0.11	0.09	2.88	2.71	5.18	5.10	0.12	0.12	0.45	0.45	0.06	0.06
6	Rep #1	2.06	2.02	1.00	1.01	0.22	0.23	0.28	0.29	0.10	0.10	2.80	2.78	5.22	5.22	0.12	0.12	0.46	0.46	0.05	0.05
	Rep #2	1.98	2.01	0.99	1.00	0.22	0.22	0.27	0.28	0.09	0.10	2.75	2.75	5.22	5.22	0.12	0.11	0.45	0.46	0.05	0.05
	□ (6)	2.02	2.02	0.99	1.00	0.22	0.23	0.28	0.29	0.09	0.10	2.78	2.77	5.22	5.22	0.12	0.12	0.45	0.46	0.05	0.05

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

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	Rep #1	2.02	2.02	0.98	0.99	0.23	0.23	0.29	0.29	0.11	0.11	2.74	2.75	5.28	5.16	0.13	0.13	0.46	0.47	0.05	0.06
7	Rep #2	2.01	2.01	0.98	0.99	0.23	0.23	0.30	0.29	0.11	0.12	2.75	2.74	5.28	5.22	0.14	0.14	0.46	0.47	0.05	0.05
	□ (7)	2.02	2.02	0.98	0.99	0.23	0.23	0.30	0.29	0.11	0.12	2.75	2.75	5.28	5.19	0.14	0.14	0.46	0.47	0.05	0.06
	Rep #1	2.09	2.11	1.02	1.01	0.24	0.24	0.29	0.29	0.10	0.09	2.83	2.84	5.20	5.25	0.10	0.10	0.47	0.46	0.05	0.05
8	Rep #2	2.10	2.09	1.00	1.01	0.24	0.23	0.29	0.29	0.10	0.10	2.80	2.78	5.18	5.18	0.10	0.08	0.47	0.47	0.05	0.05
	□ (8)	2.10	2.10	1.01	1.01	0.24	0.24	0.29	0.29	0.10	0.10	2.82	2.81	5.19	5.22	0.10	0.09	0.47	0.47	0.05	0.05
	Rep #1	1.94	2.02	1.00	1.00	0.24	0.24	0.29	0.29	0.11	0.11	2.74	2.74	5.15	5.25	0.15	0.15	0.47	0.48	0.06	0.06
9	Rep #2	1.94	2.00	1.00	1.00	0.24	0.24	0.29	0.29	0.11	0.11	2.76	2.74	5.10	5.10	0.14	0.15	0.47	0.47	0.06	0.06
	□ (9)	1.94	2.01	1.00	1.00	0.24	0.24	0.29	0.29	0.11	0.11	2.75	2.74	5.13	5.18	0.15	0.15	0.47	0.48	0.06	0.06
	Rep #1	1.95	2.05	1.00	1.00	0.23	0.23	0.29	0.29	0.11	0.10	2.70	2.65	5.20	5.30	0.14	0.14	0.48	0.45	0.05	0.05
10	Rep #2	2.00	2.00	0.99	1.00	0.23	0.25	0.28	0.29	0.10	0.11	2.70	2.75	5.40	5.30	0.13	0.13	0.48	0.46	0.05	0.05
	□ (10)	1.98	2.03	1.00	1.00	0.23	0.24	0.29	0.29	0.11	0.11	2.70	2.70	5.30	5.30	0.14	0.14	0.48	0.46	0.05	0.05
	Rep #1	0.10	<0.05	<0.05	<0.05	0.23	0.25	0.29	0.33	0.08	0.10	0.13	0.26	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.06	0.05
11	Rep #2	0.10	<0.05	<0.05	<0.05	0.25	0.24	0.28	0.27	0.08	0.10	0.13	0.26	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.05
	□ (11)	0.10	-	-	-	0.24	0.25	0.29	0.30	0.08	0.10	0.13	0.26	-	-	-	-	-	-	0.06	0.05
	Rep #1	2.49	2.47	1.17	1.16	0.29	0.27	0.38	0.38	0.14	0.13	3.51	3.42	6.37	6.12	0.18	0.18	0.61	0.60	0.06	0.05
12	Rep #2	2.56	2.53	1.16	1.20	0.30	0.28	0.38	0.37	0.14	0.13	3.43	3.32	6.28	6.24	0.18	0.18	0.61	0.60	0.06	0.05
	□ (12)	2.53	2.50	1.17	1.18	0.30	0.28	0.38	0.38	0.14	0.13	3.47	3.37	6.33	6.18	0.18	0.18	0.61	0.60	0.06	0.05
	13a	2.05	2.06	0.98	0.98	0.22	0.22	0.28	0.27	0.10	0.10	2.76	2.74	5.27	5.18	0.13	0.15	0.46	0.45	0.05	0.05
13	13b	2.05	2.06	0.98	0.98	0.22	0.22	0.28	0.27	0.10	0.10	2.76	2.74	5.27	5.18	0.13	0.15	0.46	0.45	0.05	0.05
	□ (13)	2.05	2.06	0.98	0.98	0.22	0.22	0.28	0.27	0.10	0.10	2.76	2.74	5.27	5.18	0.13	0.15	0.46	0.45	0.05	0.05
14	Rep #1	1.86	1.86	0.96	0.96	0.23	0.24	0.29	0.32	0.11	0.10	2.57	2.64	4.99	5.19	0.17	0.11	0.50	0.39	0.05	0.09
	Rep #2	1.88	1.86	0.95	0.97	0.23	0.23	0.29	0.32	0.15	0.1	2.56	2.65	5.00	5.10	0.15	0.11	0.45	0.34	0.06	0.05
	□ (14)	1.87	1.86	0.96	0.97	0.23	0.24	0.29	0.32	0.13	0.10	2.57	2.65	5.00	5.15	0.16	0.11	0.48	0.37	0.06	0.07

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

	Rep #1	1.91	1.98	1.11	1.12	0.30	0.31	0.32	0.33	0.10	0.11	3.23	3.13	5.88	6.01	0.13	0.16	0.50	0.52	0.02	0.04
15	Rep #2	1.93	1.99	1.12	1.13	0.31	0.32	0.32	0.34	0.09	0.12	3.24	3.14	5.90	6.08	0.12	0.17	0.51	0.53	0.02	0.04
	□ (15)	1.92	1.99	1.12	1.13	0.31	0.32	0.32	0.34	0.10	0.12	3.24	3.14	5.89	6.05	0.13	0.17	0.51	0.53	0.02	0.04
	Rep #1	1.98	1.99	1.00	1.00	0.23	0.23	0.28	0.28	0.10	0.10	2.78	2.82	5.27	5.34	0.13	0.13	0.47	0.47	0.05	0.05
16	Rep #2	2.04	2.08	0.99	1.00	0.23	0.23	0.28	0.28	0.10	0.10	2.79	2.81	5.30	5.28	0.14	0.14	0.47	0.47	0.05	0.05
	□ (16)	2.01	2.04	1.00	1.00	0.23	0.23	0.28	0.28	0.10	0.10	2.79	2.82	5.29	5.31	0.14	0.14	0.47	0.47	0.05	0.05
	Rep #1	2.27	2.22	1.17	1.20	0.27	0.27	0.30	0.29	0.12	0.10	2.75	2.72	5.20	4.95	0.16	0.17	0.46	0.47	0.07	0.07
17	Rep #2	2.24	2.21	1.19	1.19	0.29	0.29	0.29	0.28	0.12	0.11	2.77	2.79	5.05	4.90	0.16	0.12	0.46	0.42	0.08	0.06
	□ (17)	2.26	2.22	1.18	1.20	0.28	0.28	0.30	0.29	0.12	0.11	2.76	2.76	5.13	4.93	0.16	0.15	0.46	0.45	0.08	0.07
	Rep #1	2.08	2.08	1.02	1.00	0.24	0.23	0.29	0.28	0.11	0.10	2.80	2.82	5.40	5.37	0.14	0.15	0.48	0.49	0.06	0.05
19	Rep #2	2.08	2.08	1.01	1.00	0.23	0.24	0.28	0.29	0.10	0.11	2.82	2.80	5.28	5.40	0.14	0.14	0.47	0.47	0.05	0.05
	□ (19)	2.08	2.08	1.02	1.00	0.24	0.24	0.29	0.29	0.11	0.11	2.81	2.81	5.34	5.39	0.14	0.15	0.48	0.48	0.06	0.05

Table of data obtained. The values in italics are the results removed due to outliers from individual values according to the simple 2-tail Grubbs test and the double Grubbs test (2-tail, P = 2.5%), and according to the Cochran test (1-tail test where P = 2.5%).

Sample	A	B	C	D	E	F	G	H	I	J
Accepted labs	16	15	17	16	16	16	15	15	15	16
Repetitions	4	4	4	4	4	4	4	4	4	4
Minimum value	1.87	0.96	0.22	0.28	0.09	2.61	5.03	0.07	0.45	0.05
Maximum value	2.24	1.12	0.31	0.34	0.12	2.81	5.97	0.18	0.52	0.07
Mean value (g/L)	2.04	1.01	0.25	0.29	0.10	2.79	5.28	0.13	0.47	0.06
S <sub>r</sub>	0.03	0.01	0.01	0.01	0.01	0.05	0.08	0.01	0.01	0.01
r limit = 2 $\sigma$ * S <sub>r</sub>	0.09	0.02	0.02	0.02	0.03	0.13	0.22	0.02	0.02	0.02
RSD <sub>r</sub>	1.48%	0.76%	2.13%	1.93%	8.53%	1.70%	1.50%	3.99%	1.70%	9.86%

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S reproducibility ( $S_R$ )	0.09	0.04	0.03	0.02	0.01	0.13	0.24	0.03	0.02	0.01
R limit = $2\sigma_{2*} S_R$	0.28	0.11	0.07	0.05	0.06	0.38	0.67	0.07	0.05	0.02
RSD <sub>R</sub>	4.63%	3.96%	10.57%	5.89%	8.91%	4.81%	4.50%	19.21%	4.09%	12.49%
Horwitz RSD <sub>r</sub> (%)	3.39%	3.77%	4.66%	4.54%	5.31%	3.23%	2.94%	5.12%	4.22%	5.84%
HorRat <sub>r</sub>	0.44	0.20	0.46	0.43	1.61	0.53	0.51	0.78	0.40	1.69
Horwitz RSD <sub>R</sub> (%)	5.08%	5.65%	6.99%	6.81%	7.96%	4.85%	4.40%	7.68%	6.34%	8.75%
HorRat <sub>R</sub>	0.91	0.70	1.51	0.86	1.12	0.99	1.02	2.50	0.65	1.43

S: Standard deviation / RSD: Relative standard deviation / r: Repeatability limit / R: Reproducibility limit

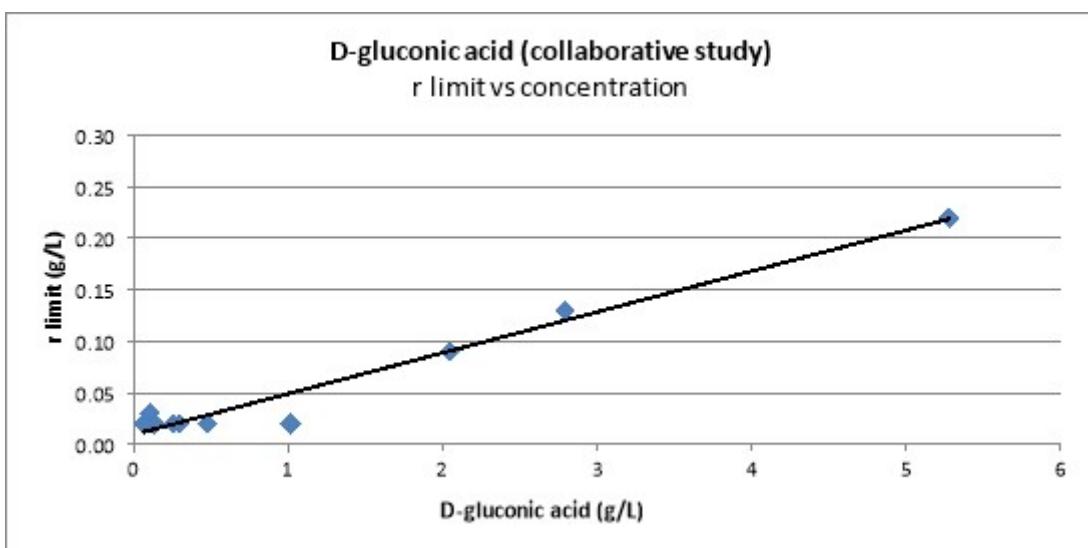


Figure 3: Repeatability limit according to concentration

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## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

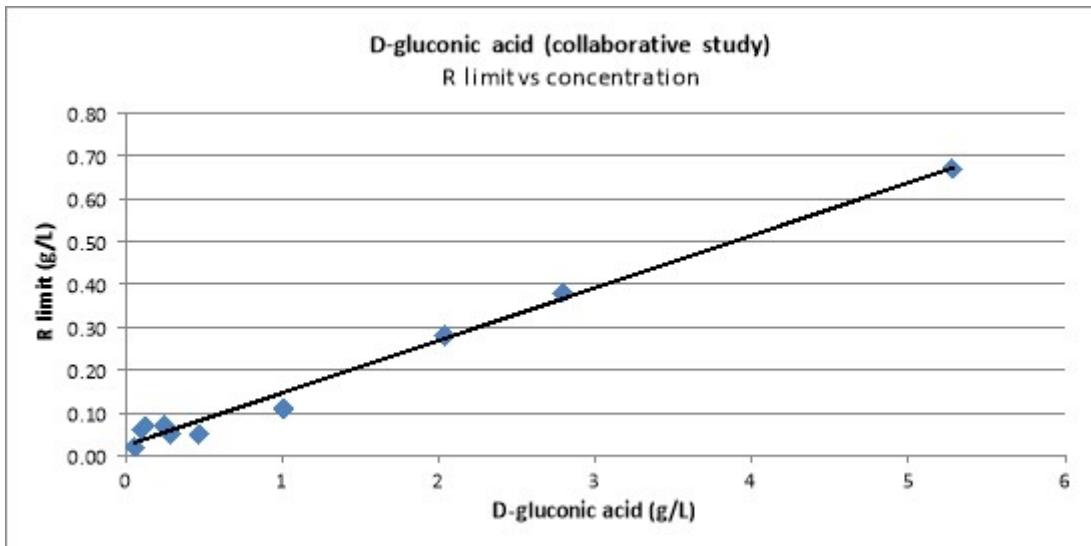


Figure 4: Reproducibility limit according to concentration

### 1.4. Manual method results

	A		B		C		D		E		F		G		H		I		J	
	1	12	2	11	3	13	4	15	5	14	6	16	7	10	8	19	9	18	17	20
Rep #1	2.05	2.09	1.06	0.99	0.25	0.25	0.34	0.33	0.10	0.12	2.85	2.84	5.32	5.34	0.14	0.13	0.45	0.46	0.05	0.05
2 Rep #2	2.08	2.10	1.03	1.02	0.23	0.26	0.35	0.32	0.09	0.10	2.83	2.86	5.34	5.36	0.15	0.13	0.44	0.45	0.05	0.05
□ (2)	2.07	2.10	1.05	1.01	0.24	0.26	0.35	0.33	0.10	0.11	2.84	2.85	5.33	5.35	0.15	0.13	0.45	0.46	0.05	0.05
Rep #1	2.24	2.11	1.01	1.04	0.26	0.26	0.34	0.33	0.11	0.11	3.05	3.19	5.64	5.68	0.14	0.16	0.34	0.41	0.05	0.05
10 Rep #2	2.37	2.24	1.01	1.06	0.25	0.26	0.35	0.34	0.12	0.11	3.10	3.02	5.65	5.78	0.14	0.15	0.33	0.42	0.05	0.05
□ (10)	2.31	2.18	1.01	1.05	0.26	0.26	0.35	0.34	0.12	0.11	3.08	3.11	5.65	5.73	0.14	0.16	0.34	0.42	0.05	0.05
Rep #1	2.61	2.54	1.04	0.99	0.27	0.28	0.34	0.34	0.13	0.12	3.44	3.38	5.97	6.22	0.21	0.23	0.44	0.47	0.05	0.05
18 Rep #2	2.57	2.54	0.97	1.01	0.28	0.28	0.35	0.35	0.12	0.12	3.32	3.42	6.04	6.31	0.21	0.21	0.51	0.53	0.05	0.05
□ (18)	2.59	2.54	1.00	1.00	0.28	0.28	0.34	0.34	0.12	0.12	3.38	3.40	6.00	6.26	0.21	0.22	0.48	0.50	0.05	0.05

Table of data obtained. The values in italics are the results removed due to outliers from individual values according to the simple 2-tail Grubbs test and the double Grubbs test (2-tail, P = 2.5%), and according to the Cochran test (1-tail test where P =

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2.5%).

Sample	A	B	C	D	E	F	G	H	I	J
Accepted labs	3	3	3	3	3	3	3	3	3	3
Repetitions	4	4	4	4	4	4	4	4	4	4
Minimum value	2.05	0.97	0.23	0.32	0.09	2.83	5.32	0.13	0.33	0.05
Maximum value	2.61	1.06	0.28	0.35	0.13	3.44	6.31	0.23	0.53	0.05
Mean value (g/L)	2.29	1.02	0.26	0.34	0.11	3.11	5.72	0.17	0.44	0.05
S <sub>r</sub>	0.06	0.02	0.01	0.01	0.01	0.02	0.11	0.01	0.03	-
r limit = $2\sqrt{2} * S_r$	0.16	0.07	0.02	0.03	0.02	0.04	0.31	0.03	0.10	-
RSD <sub>r</sub>	0.03%	0.02%	0.03%	0.03%	0.06%	0.01%	0.02%	0.06%	0.08%	-
S reproducibility (S <sub>R</sub> )	0.25	0.02	0.02	0.01	0.01	0.27	0.41	0.04	0.06	-
R limit = $2\sqrt{2} * S_R$	0.70	0.07	0.05	0.03	0.03	0.77	1.14	0.12	0.17	-
RSD <sub>R</sub>	0.11%	0.02%	0.06%	0.03%	0.10%	0.09%	0.07%	0.26%	0.14%	-
Horwitz RSD <sub>r</sub> (%)	3.33%	3.76%	4.62%	4.44%	5.24%	3.18%	2.90%	4.94%	4.27%	-
HorRat <sub>r</sub>	0.77	0.60	0.55	0.61	1.09	0.16	0.67	1.21	1.82	-
Horwitz RSD <sub>R</sub> (%)	4.99%	5.64%	6.92%	6.66%	7.86%	4.77%	4.35%	7.41%	6.41%	-
HorRat <sub>R</sub>	2.18	0.42	0.93	0.47	1.30	1.85	1.63	3.46	2.22	-

S: Standard deviation / RSD: Relative standard deviation / r: Repeatability limit / R: Reproducibility limit. The statistical parameters were calculated taking into account the results of the 3 laboratories.

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

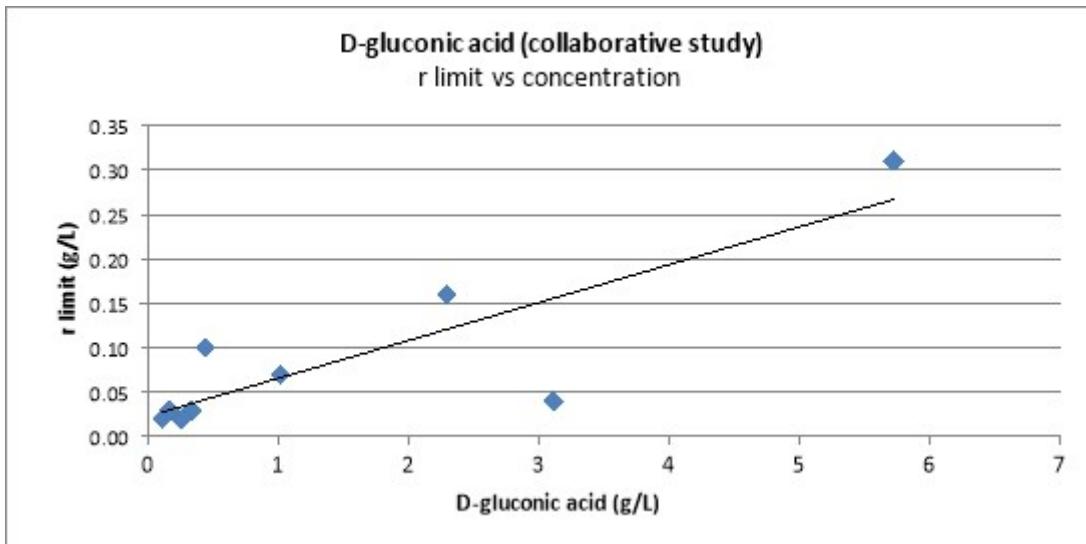


Figure 5: Repeatability limit according to concentration

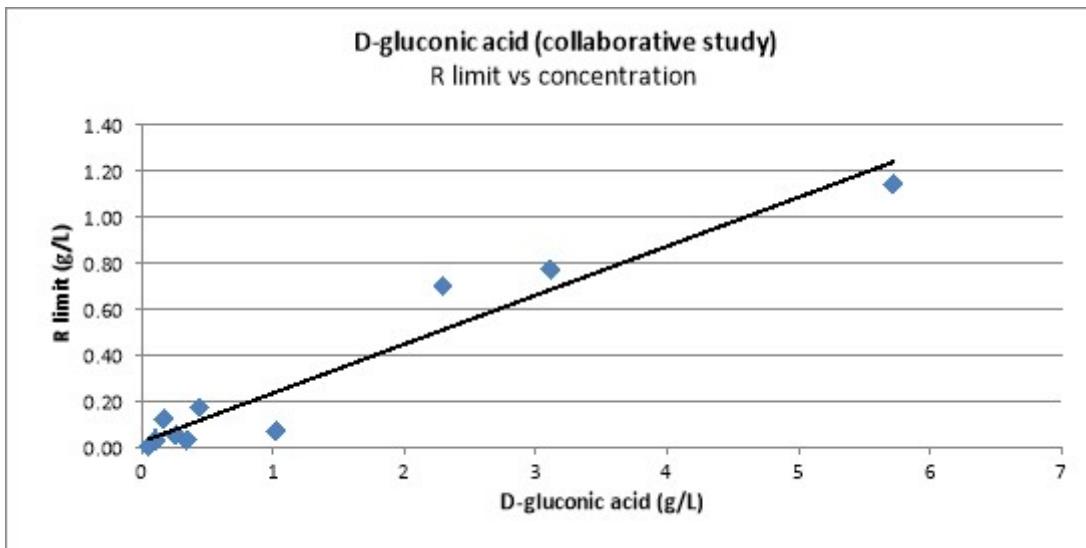


Figure 6: Reproducibility limit according to concentration

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# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Determination of D-gluconic acid in wines and musts by automated enzymatic method (Type-II)

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