RESOLUTION OENO 4/2006

DETERMINATION OF SORBIC ACID IN WINES BY CAPILLARY ELECTROPHORESIS

THE GENERAL ASSEMBLY

CONSIDERING Article 2 paragraph 2 iv of the Agreement dated April 3, 2001 establishing the International Organisation of Vine and Wine,

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

DECIDES to complete Annex A of the Compendium of International Methods of Analysis of Wine and Must by the following type IV method:

Title	Type of Method
Determination of sorbic acid in wines by capillary electrophoresis	IV

1. Scope

The present method is used to determine the sorbic acid in wines in a range from 0 to 300 mg/l.

2. Principle

The negatively charged sorbate ion naturally enables easy separation by capillary electrophoresis. At the capillary outlet, detection is carried out in the ultraviolet spectrum at 254 Nm.

3. Reagents and products

3.1. Reagents

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3.1.1. Sodium dihydrogenophosphate [10049-21-5] purity > 96%

3.1.2. Sodium hydrogenophosphate [10028-24-7] purity > 99%

3.1.3. Sodium hydroxide [1310-73-2] purity > 97%

3.1.4. Hippuric sodium [532-94-5] purity > 99%

3.1.5. Demineralised water (< 15 MOHMS) or double-distilled

3.2. Migration buffer solution

The migration buffer is made up in the following way:

- Sodium dihydrogenophosphate (3.1.1): 5 mM
- Sodium hydrogenophosphate(3.1.2):5 mM

3.3. Internal standard

Hippuric spdium (3.1.4) in an aqueous solution 0.5 g.L-1

3.4. Rinse solutions

3.4.1. Sodium hydroxide (3.1.3) N/10

3.4.2. Sodium hydroxide (3.1.3) N

4. Sample preparation

The wine samples are prepared as follows, which involves a 1/20 dilution:
Wine to be analyzed: 0.5 ml
Sodium hydroxide (3.1.3): 0.5 ml
Internal standard (3.1.4) with 0.5 g. L-1: 0.5 ml
Qsp 10 ml with demineralized water (3.1.5)

5. Operating conditions

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5.1. Conditioning the capillary

Before its first use, and as soon as the migration times increase, the capillary must be conditioned according to the following process:

5.1.1. Rinse with sodium hydroxide solution 1N (3.4.2) at 20 psi (140 kPA) for 8 min.

5.1.2. Rinse with sodium hydroxide solution (3.4.1) 0.1 N at 20 psi (140 kPA) for 12 min.

5.1.3. Rinse with water (3.1.5) at 20 psi (140 kPA) for 10 min.

5.1.4. Rinse with the migration buffer (3.2) at 20 psi (140 kPA) for 30 min.

5.2. Migration conditions

These conditions may be slightly changed depending on the equipment used.

5.2.1. The molten silica capillary is 31 cm long, with a diameter of 50 microns.

5.2.2. Migration temperature: 25°C

5.2.3. Reading wavelength: 254 nm.

5.2.4. Reading of the signal in direct mode (sorbic acid absorbs in the UV spectrum).

5.2.5. First Pre-rinse under pressure 30 psi (210 kPA) with sodium hydroxide solution 0.1 N (3.4.1) for 30 seconds

5.2.6. Second Pre-rinse under pressure 30 psi (210 kPA) with the migration buffer (3.2) for 30 seconds.

5.2.7. The injection is done under a pressure of 0.3 psi (2.1 kPA) for 10 seconds.

5.2.8. The migration lasts approximately 1.5 to 2 minutes under a potential difference of + 25 kV, in normal polarity (cathod at the exit).

5.2.9. Certain capillary electrophoresis apparatus propose large-capacity vials for migration buffer solutions. This is preferable when several analyses are carried out in series, because the electrolytic properties are maintained longer.

5.3. Reading the results

The absorption peaks for the internal standard and the sorbic acid are obtained on

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average 1 to 1.5 minutes after the start of the migration phase live. Migration time is fairly constant, but can slightly vary according to the state of the capillary. If the migration time degrades, reconditioning of the capillary is necessary, and if the nominal conditions are not restored, the capillary must be replaced.

The electrophoregram obtained is as follows:

6. Characteristics of the method

The different validation steps described were carried out according to the OIV resolution OENO 10/2005.

6.1. Intralaboratory repeatability

Standard repeatability deviation **Sr**	**1.6 mg/** L^{-1}
Repeatability **r**	**4.6 mg/** L^{-1}

6.2. Linearity

Regression line	Y = **0,99491 X + 2,52727**
Correlation coefficient **r**	**0,9997**
Residual standard deviation **Sxy**	**1,6 mg.**L^{-1}
Standard deviation slope **Sb**	**0,008 mg.**L^{-1}

6.3. Intralaboratory reproducibility

Standard reproductibility deviation **Sr**	**2.1 mg/** L^{-1}

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Reproductibility **R**	**5.8 mg/** L^{-1}

6.4. Detection and quantification limits

Detection limit **Ld**	**1.8 mg/** L^{-1}
Quantification limit **Lq**	**4.8 mg/** L^{-1}

6.5. Robustness

6.5.1. Determination

Since the method is relative, any slight variations in the analysis conditions will have no effect on the final result, but will primarily influence the migration time.

6.6. Method specificity

Possible influence of principle oenological additives were tested. None of them modify the results obtained.

6.7. Correlating the method with the OIV reference method

The OIV reference method is determination by ultraviolet absorption spectrometry. The sorbic acid, extracted by steam distillation, is determined in the wine distillate by ultraviolet absorption spectrometry at 256 Nm.

6.7.1. Comparison of repeatabilities

	Capillary electrophoresis	OIV reference method
Standard deviation of repeatability S_r	**1.6 mg/l**	**2.5 mg/** L^{-1}

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Repeatability **r**	**4.6 mg/l**	**7.0 mg/** L^{-1}

6.7.2. Accuracy of the usual method in relation to the reference method

Coefficient of correlation **r**	**0.999**
Average bias **Md**	**0.03 mg** L^{-1}
Average bias standard deviation **Sd**	**3.1 mg** L^{-1}
Z-score (Md/Sd)	**0.01**

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