RESOLUTION OENO 06/2006

DETERMINATION OF SORBIC, BENZOIC AND SALICYLIC ACID CONTENT IN WINE BY THE USE OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

THE GENERAL ASSEMBLY,

IN VIEW of article 2, paragraph 2 IV of the Agreement dated 3rd April 2001, by which the International Organization of Vine and Wine was founded,

FOLLOWING A PROPOSAL made by the Sub-commission of methods of analysis and appraisal of wines,

DECIDES to replace point 2.2.2 A of the method AS 4_02 RECANT as provided for in the Compendium of International Methods of Analysis of Wines and Musts by the following type IV method reference:

TITLE	Type of Method
Determination of sorbic, benzoic and salicylic acid content in wine by high-performance liquid chromatography	**IV**

1. Introduction

Sorbic acid and its potassium salt constitute an antiseptic that can be used in wine-making, although some countries will not tolerate even traces of it, the main reason being the smell of geraniums that develops when sorbic acid is broken down by lactic acid bacteria. Benzoic acid and salicylic acid are still prohibited in wine, but are used in other beverages.

2. Scope

All wines and grape musts, especially those likely to contain only traces of sorbic, benzoic or salicylic acid (demonstration from 1 mg/l).

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3. Principle

The antiseptics are determined using HPLC by direct injection of the sample into a column functioning by isocratic reversed-phase partition chromatography with ultraviolet detection at a wavelength of 235 nm.

4. Products

4.1. Micro-filtered fresh water (e.g. resistivity greater than 18.2 M□)

4.2. Pure tetrahydrofuran

4.3. Pure methanol

4.4. 0.1 M hydrochloric acid (prepared by means of dilution funnels)

4.5. Water with a pH of 2: adjust the pH of 650 ml of water (4.1) to pH2 using a pH meter (5.5) and by adding 0.1 M hydrochloric acid drop by drop without stirring (4.4)

4.6. Elution solution: mix 650 ml of water at pH2 (4.5) with 280 ml of methanol (4.3) and 7 ml of tetrahydrofuran (4.2)

Note: it is likewise possible to use other elution solvents, for example: 80% ammonium acetate 0.005M (0.38 g/l) adjusted to pH 4 with pure acetic acid + 20% acetonitrile.

4.7. Pure sorbic acid

4.8. Pure benzoic acid

4.9. Pure salicylic acid

4.10. Absolute alcohol

4.11. 50% vol. hydro-alcohol solution: put 500 ml of absolute alcohol (4.10) into a 1-litre flask and dilute to volume with distilled water (4.1)

4.12. Stock solution of sorbic acids at 500 mg/l: dissolve 50 mg of sorbic acids (4.7), benzoic (4.8) and salicylic (4.9) acids in 100 ml of the 50% vol. hydro-alcohol solution (4.11)

4.13. Sorbic, benzoic and salicylic acid surrogate solutions: dilute the stock solution (4.12) in the hydro-alcohol solution (4.11) in such a way as to obtain the final concentrations required. For example, for a solution of

- 200 mg/l: put 20 ml of stock solution (4.12) into a 50-ml flask and top up to the filling mark with 4.11.
- 1 mg/l: put 2 ml of stock solution (4.12) into a 50-ml flask and top up to the filling mark with 4.11.

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Intermediate solutions may be produced in the same way to satisfy calibration requirements.

5. Apparatus

5.1. Laboratory glassware, especially pipette and volumetric flasks

5.2. Ultrasonic bath

5.3. Vacuum filtration device for large volumes (1 litre) using membrane filters with a pore diameter of under 1 µm (generally 0.45 µm)

5.4. Mini-filter for samples (1 to 2 ml) using membrane filters with a pore diameter of under 1 µm (generally 0.45 µm)

5.5. pH meter

5.6. Isocratic-mode liquid phase chromatograph equipped with an injection system for small volumes (for example), 10 or 20-µl loop valve.

5.7. Detector capable of functioning at an ultraviolet rating of 235 nm and fitted with a circulating tank for HPLC (for example, 8 µl for 1 cm of optical thickness)

5.8. A 5-µm stationary phase HPLC column of the silica-type with immobilisation by octadecyl groups (C18), length 20 cm, inside diameter 4 mm

5.9. Data acquisition system

6. Preparation of samples and the elution solvent

6.1. Filter the samples to be analysed using the mini-filter (5.4)

6.2. Degas the elution solvent (4.6) for 5 minutes using the ultrasonic bath (5.2)

6.3. Filter the solvent using the device in (5.4)

7. Procedure

7.1. Column conditioning. Prior to injection, start the pump and rinse the column with the solvent for at least 30 minutes.

7.2. Inject one of the surrogate solutions (4.13) to check system sensitivity and ensure the resolution of the peaks of the substances to be analysed is satisfactory.

7.3. Inject the sample to be analysed. It is possible to analyse an identical sample, to which the acids sought have been added (adapt the amount added to the quantity observed during the previous analysis - for 1 mg present, add 1 mg, and so on).

Check the resolution of the peaks of the acids sought with the peaks of the wines

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(normally, there are none in this zone)

8. Calculation

Having located the peaks of the acids to be determined in the sample, compare the peak area with those of the acids of a surrogate solution (4.13) with a known concentration C.

For example, let s be the peak area of the acid to be determined, and S is the peak area of the solution (4.13) with concentration C

$$X_{in\ the\ sample} = C \times \frac{s}{S}\ in\ mg/l$$

9. Characteristics of the method

	Sorbic acid	Benzoic acid	Salicylic acid
Linearity range	0 to 200 mg/l	0 to 200 mg/l	0 to 200 mg/l
Accuracy (rate of recuperation)	> 90 %	> 90 %	> 90 %
Répétabilité : r*	2%	3%	8%
Reproducibility: R*	8%	9%	12%
Detection limit	3 mg/l	3 mg/l	3 mg/l
Quantification limit	5 mg/l	6 mg/l	7 mg/l
Uncertainty	11%	12%	13%

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3. Dosage de l'acide benzoic, dans les sodas et autres produits alimentaires liquides, par chromatographie en phase gazeuse. 1978. BERTRAND A. et SARRE Ch. Ann. Fals. Exp. Chim. 71, 761, 35-39.

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