RESOLUTION OIV-OENO 595-2018

DETERMINATION OF TOTAL ETHANAL IN WINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL OF the “Methods of Analysis” Sub-Commission,

DECIDES to add in Annexe A, section 3 of the Compendium of International Methods of Wine and Must Analysis the following method:

TYPE IV METHOD

DETERMINATION OF TOTAL ETHANAL IN WINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

SCOPE OF APPLICATION

The method described is suitable for the determination of total (free and sulphur-dioxide-bound) ethanal in wine for concentrations between 0.2 and 80 mg/L.

1. PRINCIPLE

The analyte is quantified after derivatisation of the molecule with 2,4-Dinitrophenylhydrazine (DNPH) followed by elution using HPLC. Detection is based on the retention time at the wavelength of 365 nm.

2. REAGENTS AND PRODUCTS

2.1. 2,4-Dinitrophenylhydrazine (DNPH), CAS no. 119-26-6, purity ≥ 99.0% (HPLC)

2.2. Sulphur dioxide (SO_2), as$K_2S_2O_5$, CAS no. 16731-55-8, purity ≥ 98%

2.3. Sulphuric acid (H_2SO_4), CAS no. 7664-93-9, purity 95.0-98.0%

2.4. Formic acid (CH_2SO_2), CAS no. 64-18-6, purity ≈ 98%

2.5. Acetonitrile (C_2H_3N), CAS no. 75-05-8, purity ≥ 99.9%

2.6. Ethanal (CH_3CHO), CAS no. 75-07-0, purity ≥ 99.5%

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2.7. Ultra-pure, HPLC-grade type I water compliant with standards ASTM D1193 and ISO 3696, CAS no. 7732-18-5

2.8. Perchloric acid ($HClO_4$), CAS no. 7601-90-3, purity 70 %

Preparation of reagent solutions

2.9. Freshly-prepared sulphur dioxide solution at a concentration of 1120 mg/L SO2, obtained by preparing a 2 g/L solution of $K_2S_2O_5$ (2.2) with ultra-pure, HPLC-grade water

2.10. 25% v/v sulphuric acid solution prepared by dilution of concentrated sulphuric acid (2.3) with ultra-pure, HPLC-grade water

2.11. 2.8% acetonitrile solution acidified with perchloric acid, obtained through the dilution of perchloric acid (2.8) in acetonitrile (2.5)

2.12. Freshly-prepared solution of 2,4-dinitrophenylhydrazine (2.1) in acidified acetonitrile (2.11) at a concentration of 2 g/L DNPH

2.13. Preparation of calibration solutions

The stock solution is prepared by dilution of an appropriate quantity of ethanal (density = 0.785 g/mL) in ultra-pure, HPLC-grade water in order to obtain a concentration of between 300 and 400 mg/L. Given that pure ethanal is highly volatile, the stock solution should be prepared by sampling constant volumes of ethanal using calibrated flasks (3.1). To prepare the stock solution, measure 10 mL pure ethanal in a calibrated flask, transfer the pure ethanal to another 20-mL calibrated flask and make up to the mark with ultra-pure, HPLC-grade water. The solutions diluted are prepared by making up to volume with ultra-pure, HPLC-grade water in calibrated flasks of greater capacity. Calibration solutions, with concentrations of 10 mg/L, 30 mg/L, 50 mg/L, 70 mg/L and 100 mg/L, are obtained through dilution of the stock solution in 50-mL calibrated flasks. The volumes required are sampled from the stock solution using a precision micropipette (3.2), for example, and made up to volume with ultra-pure, HPLC-grade water in calibrated flasks (3.1).

Preparation of solvent A for HPLC analysis

2.14. 0.5% (v/v) formic acid solution obtained by diluting concentrated formic acid (2.4) in ultra-pure, HPLC-grade water (2.7).

3. APPARATUS

3.1. Everyday laboratory glassware, including class-A calibrated flasks of 10, 20 and 50 mL, 2-mL vials and 1-L containers for the solvents, and Pasteur pipettes

3.2. Precision micropipettes

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3.3. Vortex-type stirrer

3.4. 0.45-µm membrane filters for sample preparation, certified for use in HPLC

3.5. 1-L vacuum flask (where the automatic degasser of the solvents is not provided with the HPLC apparatus)

3.6. Vacuum pump (where the automatic degasser of the solvents is not provided with the HPLC apparatus)

3.7. Analytical balance with precision of ± 0.0001 g

3.8. Natural convection oven with precision of ±1 °C at 65 °C

3.9. HPLC apparatus with UV detector, equipped with two gradient pumps and an oven for the heating of the column

3.10. C18 column (250 x 4.6 mm, particle diameter: 4 µm)

Note: Any other system may be used on the condition that the ethanal is well separated from the other derivatised carbonyl compounds. The chromatographic resolution between the ethanal peak and the greatest neighbouring peak on the chromatogram should be higher than 1.

4. SAMPLING

The wine sample should be taken and stored in a glass container sealed with a Teflon stopper in an inert atmosphere (nitrogen or argon).

5. PROCEDURE

Derivatisation

The derivatisation takes place in 2-mL glass vials sealed with Teflon stoppers, inside which the following is successively added: 100 µL wine or standard solution filtered at 0.45 µm, 20 µL freshly-prepared sulphur dioxide solution (2.2) at a concentration of 1120 mg/L SO2, 20 µL 25% sulphuric acid (2.3) and 140 µL freshly-prepared solution of 2,4-dinitrophenylhydrazine in acetonitrile at a concentration of 2 g/L DNPH (2.12).

After these additions, the solution is immediately vortex stirred and placed in the oven at 65 °C for 15 minutes before being cooled at room temperature.

Once the reaction is completed, the solution is cooled at room temperature for 15 minutes before being injected into the HPLC apparatus. The samples should be injected in less than 10 hours from the end of the derivatisation reaction.

HPLC analysis

The specific HPLC-analysis parameters are provided below by way of example.

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- Normal operating conditions:
- Injection volume: 15 µL
- Flow rate: 0.75 mL/min
- C18 column (5.10)
- Solvent for cleaning the injector: acetonitrile
- Column temperature: 35 °C
- Mobile-phase-A solvent: 0.5% formic acid in ultra-pure, HPLC-grade water
- Mobile-phase-B solvent: acetonitrile
- Detection at 365 nm

Elution gradient

The elution programme provides for:

35% eluent B (0.1 s)	65% eluent A (0,1 s)
60% eluent B (8 min)	40% eluent A (8 min)
90% eluent B (13 min)	10% eluent A (13 min)
95% eluent B (15 min)	5% eluent A (15 min)
95% eluent B (17 min)	5% eluent A (17 min)
35% eluent B (21 min)	65% eluent A (21 min)
35% eluent B (25 min)	65% eluent A (25 min)

Example chromatogram

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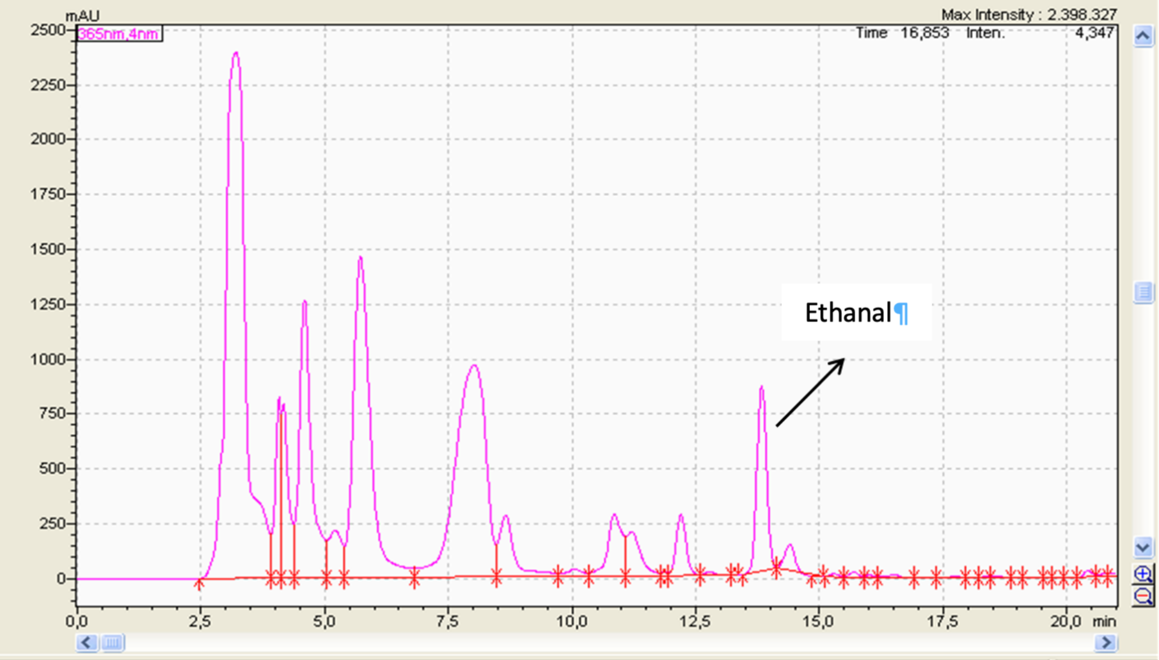

6. CALCULATION

The ethanal concentration is calculated based on the equation of the calibration curve obtained after injection of the calibration solutions (2.13).

7. PRECISION AND VALIDATION PARAMETERS

The coefficient of variation for the analyses repeated within the same laboratory should be less than 6% (for a concentration interval of between 10 mg/L and 100 mg/L). The repeatability standard deviation is 2.7% for a concentration of 14 mg/L, 2.98% for a concentration of 18 mg/L, 4.8% for a concentration of 22 mg/L and 1.3% for a concentration of 60 mg/L. The linearity range is between 0.2 and 80.0 mg/L. The limit of detection is 0.1 mg/L. The recovery rate in wine is between 92% and 102% (m/m).

8. RESULTS

The results are expressed in mg total ethanal / L to 1 decimal point.

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9. BIBLIOGRAPHY

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