RESOLUTION OIV/OENO 386B/2010

METHOD FOR THE DETERMINATION OF α-DICARBONYL COMPOUNDS OF WINE BY GASEOUS PHASE CHROMATOGRAPHY AFTER DERIVATIZATION BY 1,2-DIAMINOBENZENE

THe GENERAL ASSEMBLY

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

Upon the proposal of the Sub-commission of Methods of Analysis,

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

METHOD FOR THE DETERMINATION OF α-DICARBONYL COMPOUNDS OF WINE BY GC AFTER DERIVATIZATION BY 1,2-DIAMINOBENZENE

1. Introduction

The principal α-dicarbonyl compounds found in wine (Fig 1) are: glyoxal, methylglyoxal, diacetyl and 2,3-pentanedione, but only α-diketones are relatively abundant in wine. Carbonyl compounds exist in all types of wines, particularly after malolactic fermentation and in red wines. In addition, sweet white wines produced with botrytized grapes can contain high levels of glyoxal and methylglyoxal.

- Glyoxal: OCH−CHO (ethanedial)
- Methylglyoxal: CH_3−CO−CHO (2-oxopropanal)
- Diacetyl: CH_3−CO−CO−CH_3 (2,3-butanedione)
- 2,3-pentanedione: CH_3−CH_2−CO−CO−CH_3
- 2,3-hexanedione: CH_3−CH_2−CH_2−CO−CO−CH_3

Figure 1. The principal α-dicarbonyl compounds of wine (2,3-hexanedione is not naturally present in wine but it is used as internal standard).

Dicarbonyl compounds are important in wine for different reasons: their sensory impact, the reactivity with other components of the wine or possible microbiological effects.

2. Applicability

This method applies to all types of wines (white, red, sweetened or fortified), for carbonyl derivatives content ranging from 0.05 mg/L and 20 mg/L.

3. Principle

The method is based on the formation of derivatives of the quinoxaline type based on the □-dicarbonyl compounds of the wine with 1,2-diaminobenzene (Figure 2).

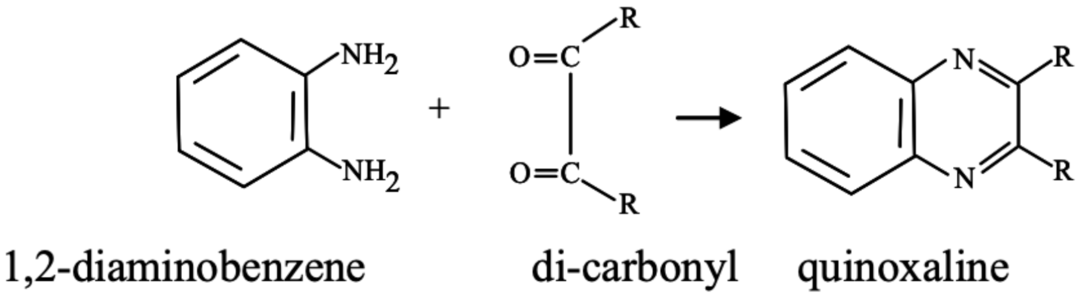

Figure 2 Formation of derivatives.

The reaction takes place directly in the wine at pH 8 and after a reaction time of 3 h at 60°C. The analysis of the derivatives is then carried out after extraction of the derivatives by dichloromethane and analysis by gas chromatography with detection by mass spectrometry (GC-MS) or using a nitrogen-specific detector.

4. Reagents and products

4.1.Dicarbonyl compounds

4.1.1. Glyoxal in a solution at 40% (CAS n° 107-22-3)

4.1.2. Methylglyoxal in a solution at 40% (CAS n° 78-98-8)

4.1.3. Diacetyl, purity > 99% (CAS n° 431-03-8)

4.1.4. 2,3-Pentanedione, purity > 97% (CAS n° 600-14-6)

4.1.5. 2,3-Hexanedione, purity > 90% (CAS n° 3848-24-6)

4.2. 1,2-Diaminobenzene in powder form, purity > 97% (CAS n° 95-54-5)

4.3. Water for HPLC (for example microfiltered and with a resistivity of 18.2 MΩ)

4.4. Pure ethanol for HPLC (CAS n° 64-17-5)

4.5. Sodium hydroxide M. (CAS n° 1310-73-2)

4.6. Sulphuric acid 2M (CAS n° 7664-93-9)

4.7. Dichchloromethane (CAS n° 75-09-2)

4.8. Anhydrous sodium sulphate (CAS n° 7757-82-6)

4.9. Aqueous-alcoholic solution at 50% vol .

Mix 50 ml of pure ethanol for HPLC (4.4) with 50 ml of water (4.3)

4.10. Solution of internal standard 2,3-hexanedione at 2.0 g/L

Place 40 mg of 2,3-hexanedione (4.2) in a 30-ml flask, dilute in 20 ml of aqueous-alcoholic solution to 50% vol (4.9) and stir until it has completely dissolved.

4.11. Anhydrous sodium sulphate (CAS n° 7757-82-6)

5. Equipment

5.1. Gas chromatograph with detection by mass spectrometry (GC-MS) or a nitrogen-specific detector.

5.1.1. Relatively polar, polyethylene glycol capillary column (CW 20M, BP21 etc.) with the following characteristics (as an example): 50 m x 0.32 mm x 0.25 µm.

5.1.2. Data acquisition system.

5.2. pH measuring apparatus

5.3. Magnetic stirrer

5.4. Balance with a precision of 0.1 mg.

5.5. Oven which can be set to 60°C

5.6. Standard laboratory glassware including pipettes, screw-cap flasks, and microsyringes.

6. Preparation of the sample

No specific preparation is necessary

7. Procedure

Place 50 ml of wine in a flask (5.6)

Bring to pH 8 while stirring, with sodium hydroxide M (4.5)

Add 25 mg of 1,2-diaminobenzene (4.2)

Add 50 µl of 2,3-hexanedione (internal standard) at 2.0 g/L (4.10)
Close the flask using a screw-cap fitted with a Teflon-faced seal
Stir until the reagent has completely disappeared (5.3)
Place in the oven at 60°C for 3 h (5.5)
Cool.

7.1. Optimisation and analytical conditions (this study was carried out by HPLC analysis, see this method)

The yield of the formation of derivatives of the dicarbonyl compounds with the 1-2-diaminobenzene is optimal at pH 8 at 60°C after three hours of reaction time
In addition, no interference of SO2 with the formation of quinoxalines was noted during the study of the method.

7.2. Analysis by GC

7.2.1. Extraction of quinoxalines

- The reaction medium prepared in 7 is brought to pH 2 using H_2SO_4 2M (4.6);
- Extract 2 times using 5 ml of dichloromethane (4.7) by magnetic stirring for 5 minutes;
- Decant the lower phase each time;
- Mix the two solvent phases;
- Dry on approximately 1 g of anhydrous sodium sulphate (4.11);
- Decant.

7.2.2. Chromatographic analysis (given as an example)

- Detection. For the analysis by GC-MS, a Hewlett Packard HP 5890 gas-phase chromatograph was coupled with Chemstation software and an HP 5970 mass spectrometer (electronic impact 70eV, 2.7 kV),

Note: It is also possible to use a nitrogen-specific detector

- Column. The column is a BP21 (SGE, 50 m x 0.32 mm x 0.25 µm).

- Temperatures. The temperature of the injector and the detector are respectively 250°C and 280°C; that of the oven is held at 60°C for 1min, then programmed to increase at a rate of 2C/min to 220°C and the final isothermal period lasts 20 min.
- Injection. The volume injected is 2 µl and the splitless time of the injector valves is 30s.

7.2.3. Analysis of quinoxalines formed

- Separation. The chromatogram of the derivatives from a wine obtained with 1,2-diaminobenzene, using selected-ion monitoring (SIM), is shown in Figure 3. Good separations were obtained with all types of wines (white, red, sweetened or fortified), and even with fermenting musts.
- Identification of the peaks. GC-MS was used to identify the dicarbonyl compounds derivatized from the wine based on the total ion current method (scan) which is used to obtain the mass spectra of derivatized quinoxalines and to compare them with those recorded in the library; in addition, the retention times were compared with those for pure compounds treated in the same way. Table 1 shows the principal ions of the mass spectra for the derivatized dicarbonyl compounds obtained.
- Determination. The quantitative determination of the dicarbonyl compounds is performed with the SIM method, by selecting ions m/z = 76, 77, 103, 117, 130, 144, 158 and 171. The ions m/z = 76 and 77 are used for the quantification and the others as qualifiers, i.e. glyoxal: ions m/z = 103 and 130, methylglyoxal: ions m/z = 117 and 144, diacetyl: ions m/z = 117 and 158, 2,3-pentandione: ions m/z = 171 and 2,3-hexanedione: ions m/z = 158 and 171.

7.2.4. Characteristics of the method

Some elements of internal validation were determined, but this is not a formal validation according to the protocol governing the planning, the implementing and the interpreting of the performance studies pertaining to the analysis methods (OIV 6/2000)

- Repeatability. The repeatability of the GC-MS-SIM method shows coefficients of variation ranging between 2 and 5% for the four dicarbonyl compounds;

- Recovery rate. The quantities added to a wine were recovered with a recovery rate ranging between 92 and 117%;
- Linearity. Linear correlations were obtained in concentrations ranging from 0.05 to 20 mg/l.
- Limit of detection. The limit of detection of most of the derivatized dicarbonyl compounds using wine as a matrix is 0.05 mg/l

Table 1. Mass spectra (ion m/z and abundance of the ion in relation to that of the base peak) of derivatives of dicarbonyl compounds using 1,2-diaminobenzene

Dicarbonyl compound	Derivative compound	Mass spectrum (principal ions and abundance
Glyoxal	Quinoxaline	130 (100), 103 (56.2), 76 (46.8), 50 (20.2), 75 (10.4), 131 (9.4)
Methylglyoxal	2-Methylquinoxaline	144 (100), 117 (77.8), 76 (40.5), 77 (23.3), 50 (21.9), 75 (11.3), 145 (10.3)
Diacetyl	2,3-Dimethylquinoxaline	117 (100), 158 (75.6), 76 (32.3), 77 (23.1), 50 (18.3), 75 (10.4)
2,3-Pentanedione	2-Ethyl-3-methylquinoxaline	171 (100), 172 (98), 130 (34.1), 75 (33.3), 77 (21), 50 (19.4), 144 (19), 143 (14.1), 103 (14)
2,3-Hexanedione	2,3-Diethylquinoxaline	158 (100), 171 (20.1), 76 (13.7), 77 (12.8), 159 (11.4), 157 (10.8), 50 (8.1)

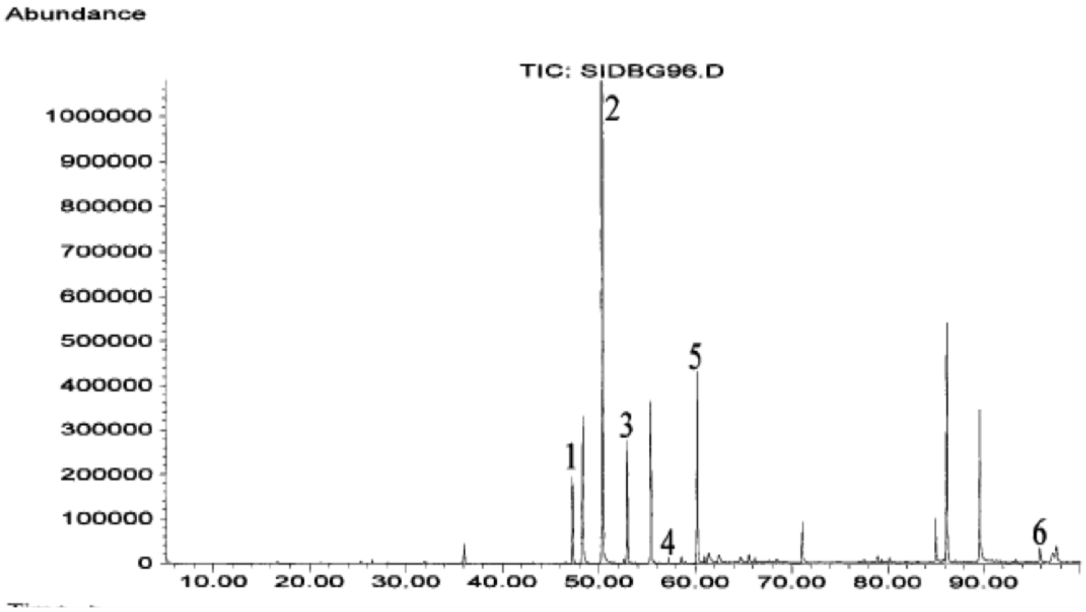

Figure 3. Gas chromatogram of the extract from the dicarbonyl compounds derivatized by 1,2-diaminobenzene from a white wine, detected by mass spectrometry by selecting the ions m/z = 76, 77, 103, 117, 130, 131, 144, 158, 160 and 171. BP21 Column, 50m x 0.32mm x 0.25 µm oven temperature 60°C for 1min, then programmed increase of 2°C/min up to 220°C. Injector temperature: 250°C. 1. glyoxal; 2. methylglyoxal; 3. diacetyl; 4. 2,3-pentanedione; 5. 2,3-hexanedione (internal standard); 6. phenylglyoxal (not studied with this method).

8. Bibliography

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