RESOLUTION OIV/OENO 382D/2010

UPDATE OF THE OIV COMPENDIUM OF METHODS OF ANALYSIS OF SPIRITOUS BEVERAGES OF VITIVINICULTURAL ORIGIN – Analysis of α-dicarbonyle compounds of spirituous beverages of viticinicultural origin by gas chromatography following 1,2-diaminobenzene derivation

THE GENERAL ASSEMBLY

IN VIEW OF article 2 paragraph 2b iv of the agreement dated 3 April 2001 by which the international organisation of vine and wine was founded,

IN VIEW OF the actions of the 2009-2012 OIV strategic plan, in particular those aiming to reorganise the publications relating to vitivinicultural methods of analysis

CONSIDERING the work of the sub-commission of methods of analysis

IN VIEW OF the « Compendium of International Methods of Analysis of Spirituous Beverages of vitivinicultural origin» adopted in 2009

HAS DECIDED to introduce the following method into the "Compendium of international methods of analysis of spirituous beverages of vitivinicultural origin"

ANALYSIS OF α-DICARBONYL COMPOUNDS IN SPIRITUOUS BEVERAGES OF VITIVINICULTURAL ORIGIN BY GAS CHROMATOGRAPHY AFTER DERIVATION BY 1,2-DIAMINOBENZENE

Type IV method

1. Introduction

The principal □-dicarbonyl compounds found in wine spirits (Figure 1) are: glyoxal, methylglyoxal, diacetyl and pentane-2,3-dione.

- Glyoxal: OCH–CHO (ethanedial)
- Methylglyoxal: CH_3–CO–CHO (2-oxopropanal)
- Diacetyl: CH_3–CO–CO–CH_3 (butane-2,3-dione)
- Pentane-2,3-dione: CH_3–CH_2–CO–CO–CH_3

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- Hexane-2,3-dione: $CH_3-CH_2-CH_2-CO-CO-CH_3$

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

Dicarbonyl compounds are important because of their sensory impact.

2. Applicability

This method applies to spirituous beverages of vitivinicultural origin, for a content of carbonyl compounds included between 0.05 mg/L and 20 mg/L.

3. Principle

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

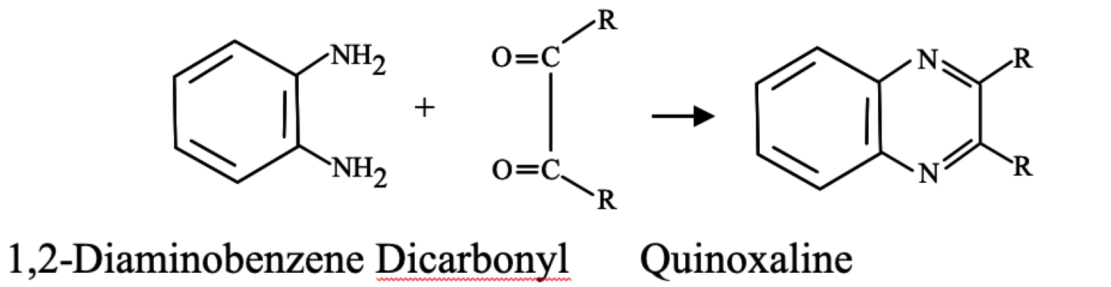

Figure 2 Formation of derivatives.

The reaction takes place in the spirituous beverage diluted four-fold, pH 8 and after a reaction time of 3 hrs 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 specific detector of nitrogenous compounds.

4. Reagents and products

4.1. Dicarbonyl compounds

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

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

4.1.3. Diacetyl (CAS N° 431-03-8) > 99 % pure

4.1.4. Pentane-2,3-dione (CAS N° 600-14-6) > 97 % pure

4.1.5. Hexane-2,3-dione (CAS N° 3848-24-6) > 90 % pure

4.2. 1,2-Diaminobenzene (CAS N° 95-54-5) in the forme of powder, > 97 % pure

4.3. Water for HPLC (according to standard EN ISO 3696)

4.4. Ethanol (CAS N° 64-17-5) pure for HPLC

4.5. Sodium Hydroxide (CAS N° 1310-73-2) in a 0,1M solution

4.6. Acetic acid (CAS N° 7664-93-9) pure cristallisable

4.7. Dichloromethane (CAS N° 75-09-2)

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

4.9. 50% vol. hydroalcoholic solution.

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

4.10. Solution of internal standard hexane-2,3-dione at 2.0 g/l

Place 40 mg of hexane-2,3-dione (4.2) in a 30 ml flask, dilute in 20 ml of 50% vol. hydroalcoholic solution. (4.9), stir until complete dissolution.

5. Apparatus

5.1. Gas chromatography with detection by mass spectrometry (GC-MS) or using a special nitrogenised compound detector.

5.1.1. Moderately polar, polyethylene glycol capillary column (such as the Carbowax 20M, BP21) 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. 0.1 Mg analytical balance

5.5. Oven which can be set to 60°C

5.6. Standard laboratory glassware including pipettes, 30-ml screw-cap flasks, and microsyringes.

6. Preparation of the sample

Dilute the spirituous beverage four-fold in water (4.3)

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7. Procedure

Place 10 ml of spirituous beverage diluted four-fold (6) in a 30 ml flask
Bring to pH 8 while stirring, with sodium hydroxide 0.1 M (4.5)
Add 5 mg of 1,2-diaminobenzene (4.2)
Add 10 µl of hexane-2,3-dione (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 hrs (5.5)
Cool

7.1. Analysis

7.1.1. Extraction of quinoxalines

- The reactional medium prepared at 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;
- Elutriate the lower phase each time;
- Mix the two solvent phases;
- Dry on approximately 1 g of anhydrous sodium sulphate (4.8);
- Elutriate.

7.1.2. Chromatographic analysis (given as an example)

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

Note: A specific detector of the nitrogenous compounds can be used.

- Column. The column is a BP21 (SGE, 50 m x 0.32 mm x 0.25 µm).
- Temperatures. The temperatures of the injector and the detector are 250°C and

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280°C, respectively; the oven is kept at 60°C for 1 min., then programmed to increase at a rate of 2C/min to 220°C, and the final isotherm lasting 20 min.

- Injection. The volume injected is 2 µl and the closing time of the injector valves (splitless) is 30s.

7.1.3. Analysis of quinoxalines formed

- Separation. The chromatogram of the derivatives by 1,2-diaminobenzene of a wine according to the ion selection method (SIM) is shown in Figure 3.
- Identification of the peaks. GC-MS was used to identify the dicarbonyl compounds derived from the wine spirit based on the total ionic current method (scan) which is used to obtain the mass spectra of quinoxaline derivatives and to compare them with those memorised in the spectra 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 obtained dicarbonyl compound derivates.
- Proportioning. The quantitative determination of the dicarbonyl compounds is carried out 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, pentan-2,3-dione: ions M/Z = 171 and hexane-2,3-dione: ions M/Z = 158 and 171.

7.1.4. Characteristics of the method

Some internal validation were defined but these are not a formal validation as per the protocol governing the planning, the implementing and the interpreting of the performance studies relating to the analysis methods (OIV 6/2000)

- Repeatability. The repeatability of the GC-MS-SIM method displays variation coefficients ranging between 2 and 5% for the four dicarbonyl compounds;
- Recovery rate. The quantities added to a wine were recovered with with a below 6% deviation from expected results;
- Linearity. Linear correlations were obtained in concentration domains ranging between 0.05 to 20 mg/l.

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- Detection limit. The detection limit of most of the derived dicarbonylated products is 0.05 mg/l.

Table 1. Mass spectra (ion m/z (intensity of the molecular ion in relation to that of the basic peak) of derivatives of dicarbonyl compounds by 2,3-diaminobenzene

Dicarbonylated 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)
Pentane-2,3-dione	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)
Hexane-2,3-dione	2,3-Diethylquinoxaline	158 (100), 171 (20.1), 76 (13.7), 77 , (12.8), 159 (11.4), 157 (10.8), 50 (8.1)

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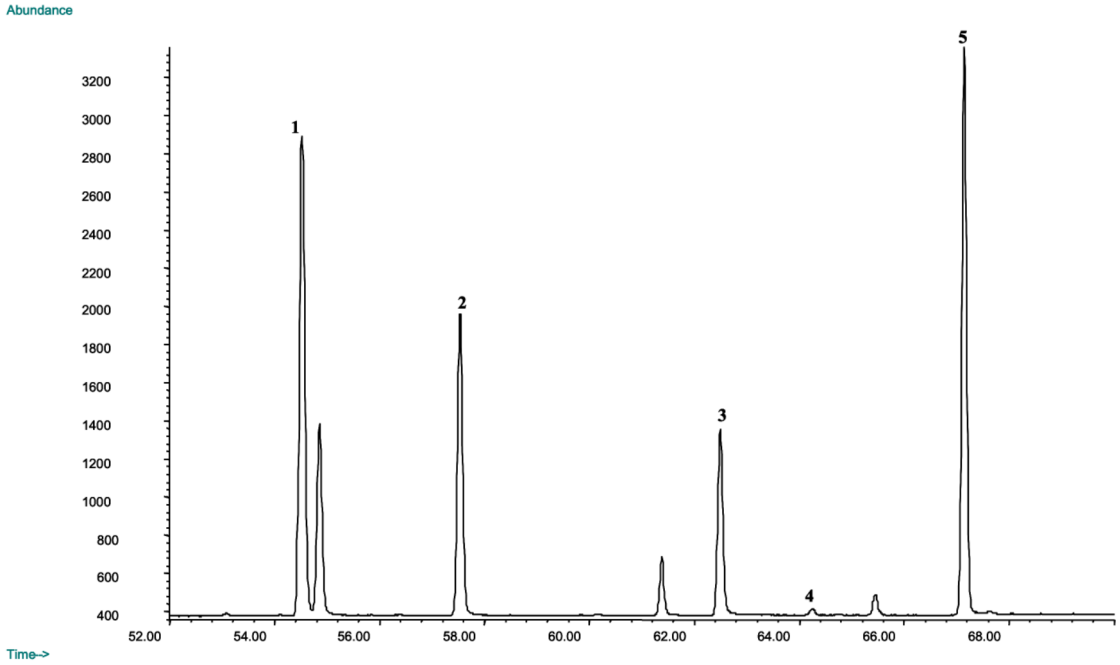

Figure 3. High-performance liquid phase chromatogram of dicarbonyl compounds derived by 1,2-diaminobenzene from wine spirit, detected through mass spectrometry by selecting the ions m/z = 76, 77, 103, 117, 130, 131, 144, 158, 160 and 171. BP21 column, 50m _ 0.32mm 0.25 µm oven Temperature 60 ° C for 1 min, then programmed to increase 2 ° C/min up to 220 ° C. injector Temperature: 250° C. 1. glyoxal; 2. methylglyoxal; 3. diacetyl; 4. pentane-2,3-dione; 5. hexane-2,3-dione (internal standard); 6. phenylglyoxal (not determined for this method).

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