OIV-MA-BS-18 Analysis of □ -dicarbonyl compounds in spiritous beverages of viti-vinicultural origin by HPLC after derivation by 1,2 diaminobenzene

Type IV method

1. Introduction

The principal □-dicarbonyl compounds found in wine-based 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
Hexane-2,3-dione	CH_3–CH_2–CH_2–CO–CO–CH_3

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

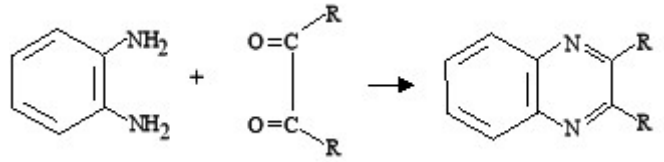
Dicarbonyl compounds are important because of their sensory impact,

2. Applicability

This method applies to spirituous beverages of vitivinicultural origin for dicarbonyl compounds with a content ranging 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).



1,2-Diaminobenzene Dicarbonyl Quinoxaline

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 either directly by chromatography in the high-performance liquid phase (HPLC) and detection by UV absorptiometry at 313 Nm,.

4. Reagents and products

1. Dicarbonyl compounds
    1. Glyoxal (CAS N° 107-22-3) in a 40% solution
    2. Methylglyoxal (CAS N° 78-98-8) in a 40% solution
    3. Diacetyl (CAS N° 431-03-8) > 99 % pure
    4. Pentane-2,3-dione (CAS N° 600-14-6) > 97 % pure
    5. Hexane-2,3-dione (CAS N° 3848-24-6) > 90 % pure
2. 1,2-Diaminobenzene (CAS N° 95-54-5) in the form of powder, > 97 % pure
3. Water for HPLC (according to standard EN ISO 3696)
4. Ethanol (CAS N° 64-17-5) pure for HPLC
5. Sodium Hydroxide (CAS N° 1310-73-2) in 0.1M solution
6. Acetic acid (CAS N° 64-19-7) pure crystallisable
7. Solvent A for the analysis by HPLC

In 1 water L for HPLC (4.3), add 0.5 ml of acetic acid (4.8), mix, degas (by ultrasound, for example)

4.8. Solvent B for HPLC

Pure HPLC methanol (CAS N° 67-56-1)

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. (4.11), stir until complete dissolution.

5. Apparatus

1. High-performance liquid phase chromatography with detection by UV absorption (313 nm);
    1. Analytical column filled with silica grafted by octadecyl radicals of 5 µm with dimensions of 250 mm x 4.6 mm, for example.
    2. Data acquisition system.
2. pH measuring apparatus
3. Magnetic stirrer
4. Mg analytical balance
5. Solvent degasification system for HPLC (an ultrasound apparatus, for example)
6. Oven which can be set to 60°C
7. Standard laboratory glassware including pipettes, 30-ml (5.7) screw-cap flasks, and microsyringes.

6. Preparation of the sample

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

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 2,3-diaminobenzene (4.2)

Add 10 µl of hexane-2,3-dione (internal standard) at 2.0 g/1 (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 (5.6)

Cool.

7.1. Analysis

Injection. After cooling, the reactional medium containing the quinoxalines is directly injected into the HPLC system at an amount of 20 µl.

- *Elution programme.* For separation, an example of an elution schedule is displayed in Table 1

Table 1. Example of HPLC analysis elution schedule

Time in minutes	Solvent A	Solvent B
0	80	20
8	50	50
26	25	75
30	0	100
32	0	100

The flow rate being 0.6 ml/min

- *Separation.* The chromatogram obtained by HPLC is shown in Figure 3
- *Detection*. The maximum absorption was studied for all the dicarbonyl compound derivatives and set at 313 Nm as being optimal.
- *Identification of the derivatives.* The identification of the derivatives was carried out by comparing the retention times with standard reference solutions. The chromatographic conditions enable a good separation of the peaks in all the wines.

7.1.1. Characteristics of the method

Some internal validation elements were determined but these are not a formal validation according the protocol for the planning, the implementing and the interpretation of the performance studies pertaining to analysis methods (OIV

6/2010)

- *Linearity*. The linearity of the method was tested using standard solutions (the hydroalcoholic solution at 12% vol. was used as a matrix) (Table 2). The quantitative analysis of the additions of dicarbonyl compounds showed that the method is linear for the four compounds with recovery rate varying between 92 and 117%.

Table 2. Study of the linearity and recovery tests with standard solutions (12% v/v water-ethanol) correlation coefficients

Glyoxal	Methylglyoxal	Diacetyl	Pentane-2,3-dione
value[a] surface[b]	value[a] surface[b]	value[a] surface[b]	value[a] surface[b]
R=0,992	R=0,997	R=0,999	R=0,999

a: mg/l, b: arbitrary units, c: response factor in relation to the internal standard.

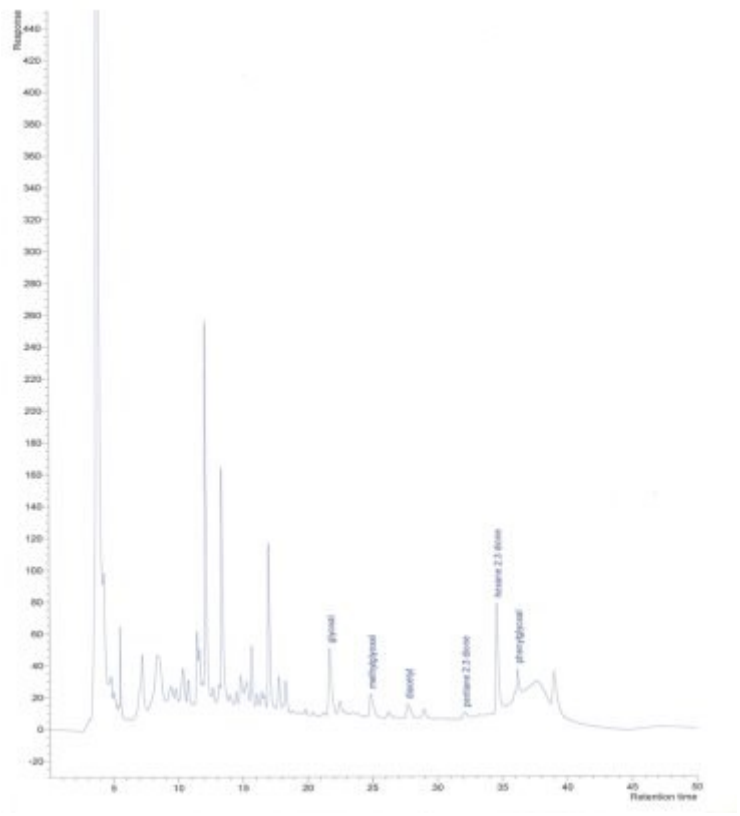
- *The quantification limit* of the dicarbonyl compounds is very low, the best results being obtained with diacetyl, the detection limit of which is 10 times weaker than that of the other compounds (table 3).

Tableau 3. Performance of the HPLC method for the quantification of dicarbonyl compounds

Limits	detection[a]	determination[a]	quantification[a]
Glyoxal	0,015	0,020	0,028
Methylglyoxal	0,015	0,020	0,027
Diacetyl	0,002	0,002	0,003
Pentane-2,3-dione	0,003	0,004	0,006

a: results in mg/L, hydroalcoholic solution (10% vol.).

Figure 3. High-performance liquid phase chromatogram of dicarbonyl compounds derivatized by 1,2-diaminobenzene, detected by UV at 313 nm. Spherisorb ODS Column 250 mm x 4.6 mm x 5 µm.



8. Bibliography

- Bartowski E.J.and Henschke P.A. The buttery attribute of wine – diacetyl – desirability spoilage and beyond. Int. j.food microbial. 96 : 235-252 (2004).
- Bednarski W, Jedrychowski L, Hammond E and Nikolov L, A method for determination of □-dicarbonyl compounds. J Dairy Sci 72:2474-2477 (1989).
- Leppannen O, Ronkainen P, Koivisto T and Denslow J, A semiautomatic method for the gas chromatographic determination of vicinal diketones in alcoholic beverages. J Inst Brew 85:278- 281 (1979).
- Martineau B, Acree TE and Henick-Kling T, Effect of wine type on the detection threshold for diacetyl. Food Res Int 28:139-143 (1995).

- Moree-Testa P and Saint-Jalm Y, Determination of □-dicarbonyl compounds in cigarette smoke. J Chromatogr 217:197-208 (1981).
- de Revel G Pripis-Nicolau L. Barbe J.-C; and Bertrand A, The detection of α-dicarbonyl compounds in wine by formation of quinoxaline derivatives. J Sci. Food Agric.80:102-108 (2000).
- de Revel G and Bertrand A, Dicarbonyl compounds and their reduction products in wine. Identification of wine aldehydes. Proc 7th Weurman Flavour Research Symp, Zeist, June, pp 353-361 (1994).
- de Revel G and Bertrand A, A method for the detection of carbonyl compounds in wine: glyoxal and methylglyoxal. J Sci. Food Agric 61:267-272 (1993).
- Voulgaropoulos A, Soilis T and Andricopoulos N, Fluorimetric determination of diacetyl in wines after condensation with 3,4-diaminoanisole. Am J Enol Vitic 42:73-75 (1991).