

# COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS FOR SPIRITUOUS BEVERAGES AND ALCOHOLS

OIV-MA-BS-18 Analysis of alpha-dicarbonyl compounds by HPLC after derivation by 1,2-diaminobenzene in spirit drinks of viti-vinicultural origin  
Method OIV-MA-BS-18 : R2010

Type IV method

## Analysis of $\alpha$ -dicarbonyl compounds in spiritous beverages of viti-vinicultural origin by HPLC after derivation by 1,2-diaminobenzene

(OIV/OENO 382C/2010)

### 1. Introduction

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

Glyoxal	OCH-CHO (ethanedial)
Methylglyoxal	CH <sub>3</sub> -CO-CHO (2-oxopropanal)
Diacetyl	CH <sub>3</sub> -CO-CO-CH <sub>3</sub> (butane-2,3-dione)
Pentane-2,3-dione	CH <sub>3</sub> -CH <sub>2</sub> -CO-CO-CH <sub>3</sub>
Hexane-2,3-dione	CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CO-CO-CH <sub>3</sub>

*Figure 1. The principal  $\alpha$ -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;

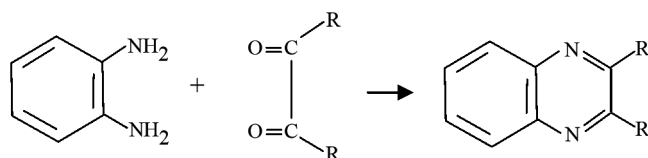
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### 3. Principle

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The method is based on the formation of quinoxaline derivatives from  $\alpha$  - 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

#### 4.1. Dicarbonyl compounds

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

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

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**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 0.1M solution**

**4.6. Acetic acid (CAS N° 64-19-7) pure crystallisable**

**4.7. 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**

**5.1. High-performance liquid phase chromatography with**

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**detection by UV absorption (313 nm);**

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**5.1.1. Analytical column filled with silica grafted by octadecyl radicals of 5 µm with dimensions of 250 mm x 4.6 mm, for example.**

**5.1.2. Data acquisition system.**

**5.2. pH measuring apparatus**

**5.3. Magnetic stirrer**

**5.4. Mg analytical balance**

**5.5. Solvent degasification system for HPLC (an ultrasound apparatus, for example)**

**5.6. Oven which can be set to 60°C**

**5.7. 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/l (4.10)

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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

<i>Table 1. Example of HPLC analysis elution schedule</i>		
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.

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## OIV-MA-BS-18 Analysis of alpha-dicarbonyl compounds by HPLC after derivation by 1,2-diaminobenzene in spirit drinks of viti-vinicultural origin

### 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%.

<i>Table 2. Study of the linearity and recovery tests with standard solutions (12% v/v water-ethanol) correlation coefficients</i>			
Glyoxal	Methylglyoxal	Diacetyl	Pentane-2,3-dione
value <sup>a</sup> surface <sup>b</sup>	value <sup>a</sup> surface <sup>b</sup>	value <sup>a</sup> surface <sup>b</sup>	value <sup>a</sup> surface <sup>b</sup>
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).

<i>Tableau 3. Performance of the HPLC method for the quantification of dicarbonyl compounds</i>			
Limits	detection <sup>a</sup>	determination <sup>a</sup>	quantification <sup>a</sup>
Glyoxal	0,015	0,020	0,028

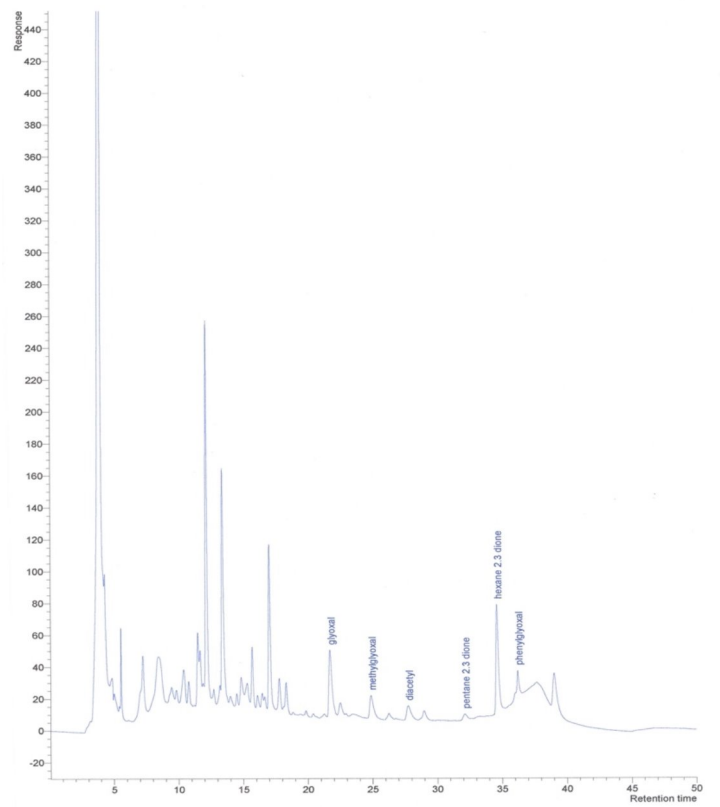
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Methylglyoxal	0,015	0,020	0,027
Diacetyl	0,002	0,002	0,003
Pentane-2,3-dione	0,003	0,004	0,006

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

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