**Method OIV-MA-BS-18 : R2010**

**Type IV method**

# Analysis of  -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 -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).* | |

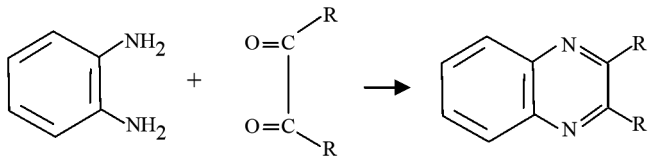
Dicarbonyl compounds are important because of their sensory impact,

## 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

## 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

## 4.2. 1,2-Diaminobenzene (CAS N° 95-54-5) in the form 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 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

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

**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/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).

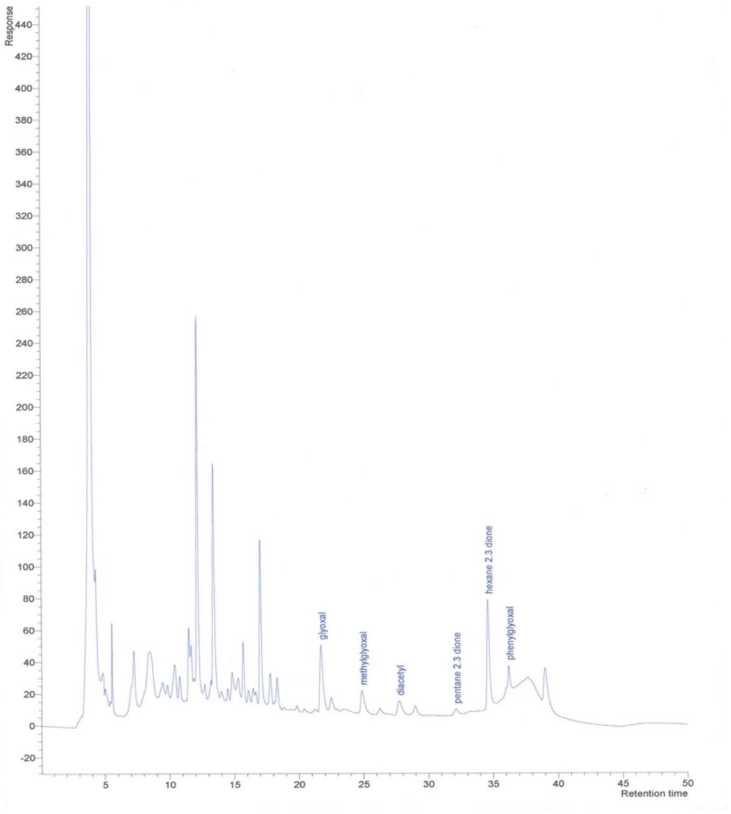
*Tableau 3. Performance of the HPLC method for the quantification of dicarbonyl compounds*

| 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

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