OIV-MA-AS315-20 Method for the determination of □-dicarbonyl compounds of wine by HPLC after derivizationation by 1,2-diaminobenzene

Type IV method

1. Introduction

The principal □-dicarbonyl compounds found in wine (Fig 1) are: glyoxal, methylglyoxal, diacetyl and pentane-2,3-dione, but only □-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 □-dicarbonyl compounds of wine (2,3-hexanedione is not naturally present in wine but it is used as internal standard).

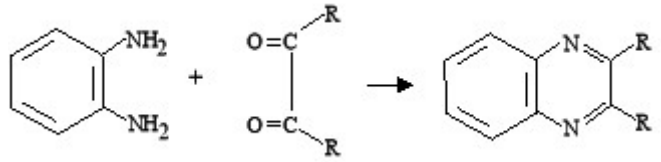
Dicarbonyl compounds are important in wine for different reasons: their sensory impact, the reactivity with other components of the wine or possible microbiological effects.

2. Applicability

This method applies to all types of wines (white, red, sweetened or fortified), for dicarbonyl compounds with a content that ranges from 0.05 mg/l to 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).



1,2-diaminobenzene dicarbonyl quinoxaline

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 directly by high-performance liquid chromatography (HPLC) and detection by UV absorption at 313 nm.

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%

4.3. Water for HPLC (for example microfiltered and with a resistivity of 18.2 MΩ) (CAS N° 95-54-5)

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

4.5. Sodium Hydroxide M (CAS N° 1310-73-2)

4.6. Pure crystallisable acetic acid (CAS N° 64-19-7)

4.7. Solvent A for the analysis by HPLC

To 1 l of water for HPLC (4.3) add 0.5 ml of acetic acid (4.8), mix, degas (for example by sonication)

4.8. Solvent B for HPLC

Pure methanol for HPLC (CAS N° 67-56-1)

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.

5. Equipment

1. High-performance liquid chromatograph with detection by UV absorption (313 nm);
    1. Analytical column filled with 5 µm octadecyl silica whose dimensions are for example 250 mm x 4.6 mm.
    2. Data acquisition system.
2. pH measuring apparatus.
3. Magnetic stirrer.
4. Balance with a precision of 0.1 mg.
5. Solvent degasification system for HPLC (for example an ultrasonic bath).
6. Oven which can be set to 60°C.
7. Standard laboratory glassware including pipettes, 30-ml screw-cap flasks, and microsyringes.

6. Preparation of the sample

No specific preparation is necessary.

7. Procedure

- Place 10 ml of wine in a 30-ml flask (5.7)
- Bring to pH 8 while stirring, with sodium hydroxide M (4.5)
- Add 5 mg of 1,2-diaminobenzene (4.2)
- Add 10 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 (5.6)
- Cool.

7.1. Optimisation and analytical conditions

The yield of the reaction of the dicarbonyl compounds with the 1-2-diaminobenzene is

optimal at pH 8. Solutions of dicarbonyl compounds have been derivatized at 25, 40 or 60°C and then analysed by HPLC according to the protocol described in point 7.2 at different times (Table 1). Diketones require much more reaction time and a higher reaction temperature. The reaction is slower with molecules with longer chains (2,3-pentanedione and 2,3-hexanedione).

In addition, no interference of SO_2 with the formation of quinoxalines was noted during the study of the method.

Table 1. Effect of reaction time and temperature on the formation of derivatives by diaminobenzene from glyoxal, diacetyl and 2,3-hexanedione

		Reaction time		
		1h	2h	3h
	Temperature (°C)	Recovery rate (%)		
Glyoxal	25	92	93	94
	40	95	97	98
	60	96	98	100
Diacetyl	25	23	77	87
	40	64	89	94
	60	85	100	100
2,3-Hexanedione	25	17	67	79
	40	55	79	88
	60	69	93	100

Table 1. Effect of reaction time and temperature on the formation of derivatives by diaminobenzene from glyoxal, diacetyl and 2,3-hexanedione

7.2. Analysis by HPLC

- *Injection*. After cooling, 20 µl of the reaction medium containing the quinoxalines is directly injected into the HPLC system.

- *Elution programme*. For the separation, the elution programme is presented in Table 2

Table 2. Elution programme for the analysis by HPLC

Time in minutes	Solvent A	Solvent B
0	80	20
8	50	50
26	25	75
30	0	100
32	0	100
40	100	0
45	80	20
50	80	20

Table 2. Elution programme for the analysis by HPLC

The flow rate is 0.6 ml/min

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

1. Characteristics of the method by HPLC

Some internal validations methods have been determined but do not constitute a formal validation proccess according to the protocol governing the planning, the implementing and the interpreting of performance studies pertaining to analysis methods (OIV 6/2000)

Repeatability. The repeatability of the method was calculated using 10 analyses of the

same wine (Table 3).

	Average*	Standard deviation	CV(%)
White wine			
Glyoxal	4.379	0.101	2.31
Methylglyoxal	2.619	0.089	3.43
Diacetyl	5.014	0.181	3.62
2.3-Pentanedione	2.307	0.0097	4.21
Red wine			
Glyoxal	2.211	0.227	10.30
Methylglyoxal	1.034	0.102	9.91
Diacetyl	1.854	0.046	2.49
2,3-Pentanedione	0.698	0.091	13.09

Table 3. Repeatability study and performance of the method

* Results in mg/l based on 10 analyses of the same wine.

Linearity. The linearity of the method was tested using standard solutions (using an aqueous-alcoholic solution at 12% vol. as a matrix) (Table 4). The quantitative analysis of the additions of dicarbonyl compounds showed that
the method is linear for the four compounds and that its precision is satisfactory.

Glyoxal	Methylglyoxal	Diacetyl	Pentane-2,3-dione
value[a] peak area [b]	value[a] peak area [b]	value[a] peak area [b]	value[a] peak area [b]
1			
R = 0.992	R = 0.997	R = 0.999	R = 0.999

Table 4. Study of the linearity and recovery tests with standard solutions (water-ethanol at 12% v/v) Value of the correlation coefficient

The recovery of additions carried out in red and white wines demonstrated the satisfactory performance of the method . Contained in the 92% - 116% range for extreme values

The quantification limit of the dicarbonyl compounds is very low, the best results being obtained with diacetyl, whose detection limit is 10 times lower than that of the other compounds (Table 5).

Limits	detection[a]	determination[a]	quantification[a]
Glyoxal	0.015	0.020	0.028
Methyglyoxal	0.015	0.020	0.027
Diacetyl	0.002	0.002	0.003
2.3-Pentanedione	0.003	0.004	0.006

able 5. Performance of the method by HPLC for the quantification of dicarbonyl compounds

a: results in mg/l, aqueous-alcoholic solution (10% vol).

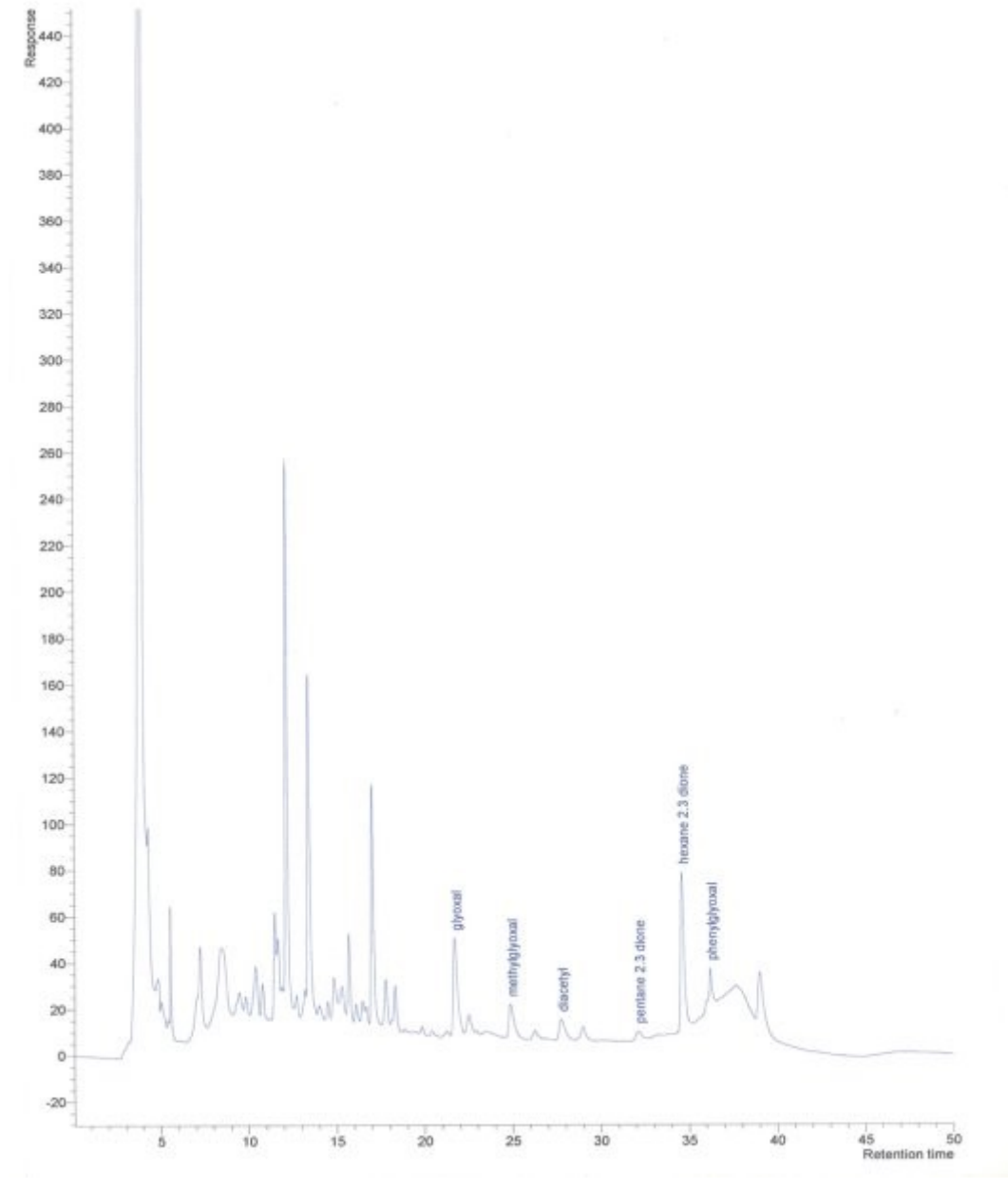


Figure 3. High-performance liquid phase chromatogram of dicarbonyl compounds derivatized by 1,2-diaminobenzene from a white wine, detected by UV at 313 nm. Spherisorb ODS Column 250 mm x 4.6 mm x 5 µm.

Bibliography

- Bartowski E.J. and Henschke P.A., The buttery attribute of wine – diacetyl –

desirability spoilage and beyond. Int. J. Food Microbiol. 96: 235-252 (2004).

- Bednarski W., Jedrychowski L., Hammond E., and Nikolov L., A method for determination of α-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 T. 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 the 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).
- Gilles de Revel et Alain Bertrand Analyse des composés α-dicarbonyles du vin après dérivation par le 2,3-diaminobenzène OIV FV 1275