

## **OIV-MA-AS313-12B Determination of d-malic acid in wines at low concentrations using the enzymatic method**

Type IV method

### **1. Field of application**

The method described is applied to dosage, by the enzymatic means, of malic acid D of wines with contents under 50 mg/l.

### **2. Principle**

The principle of the method is based on malic acid D(+) oxidation (D-malate) by nicotinamide-adenine-dinucleotide (NAD) in oxaloacetate that is transformed into pyruvate and carbon dioxide; the formation of NADH, measured by the increase of absorbance in wave length at 340 nm, is proportional to the quantity of D-malate present (principle of the method described for malic acid D determination for concentrations above 50 mg/l), after introducing a quantity of malic acid D of 50 mg/l in a cuvette.

### **3. Reagents**

Malic acid D solution of 0.199 g/l, above reagents indicated in the methods described for contents above 50 mg/l.

### **4. Apparatus**

Apparatus indicated in the method described for concentration above 50 mg/l.

### **5. Sample preparation**

Sample preparation is indicated in the method described for concentrations above 50 mg/l.

### **6. Procedure**

The procedure is indicated in the method described for concentrations above 50 mg/l. (Resolution Oeno 6/98), but with the introduction in the tank of a quantity of malic acid D equivalent to 50 mg/l. (Introduction of 0.025 mL of malic acid D at 0.199 g/l, substituting the equivalent volume of water); the values obtained are decreased by 50 mg/l.

### **7. Internal validation**

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## D-malic Acid: enzymatic method low concentrations (Type-IV)

Summary of the internal validation file on the dosage of malic acid D(+)-after the addition of 50 mg/l of this isomer

|                               |   |
|-------------------------------|---|
| Work level                    | 0 mg of 70 mg of malic acid D(+)-per liter.<br>Within these limits, the method is linear with a correlation coefficient between 0.990 and 0.994 |
| Setting limit                 | 24.4 mg/l   |
| Detection limit               | 8.3 mg/l  |
| Sensitivity                   | 0.0015 abs / mg/l   |
| Recovery percent range        | 87.5 to 115.0% for white wines and 75 to 105% for red wines   |
| Repeatability                 | =12.4 mg/l for white wines<br>(according to the OIV method =12,5 mg/l)<br>=12.6 mg/l for red wines<br>(according to OIV method=12,7 mg/l)       |
| Percentage standard deviation | 4.2% to 7.6% (white wines and red wines)  |
| Intralaboratory variability   | CV=7.4% (s=4.4mg/l; X average=59.3 mg/l)  |

### 8. Bibliography

- Chretien D., Sudraud P., 1993. Présence naturelle d'acide D(+)-malique dans les moûts et les vins, *Journal International des Sciences de la Vigne et du Vin*, 27: 147-149.
- Chretien D., Sudraud P., 1994. Présence naturelle d'acide D(+)-malique dans les moûts et les vins, *Feuille Vert de l'OIV*, 966.
- Delfini C., Gaetano G., Gaia P., Piangerelli M.G., Cocito C., 1995. Production of D(+)-malic acid by wine yeasts, *Rivista de Viticoltura e di Enologia*, 48: 75-76.
- OIV, 1998. *Recueil des méthodes internationales d'analyse des vins et des moûts. Mise à jour Septembre 1998*. OIV, Paris.

- Przyborski H., Wacha C., Bandion F., 1993. Zur bestimmung von D(+)Apfelsäure in wein, *Mitteilung Klosterneuburg*, 43: 215-218.
- Machado M. and Curvelo-Garcia A.S., 1999; FV.O.I.V. N° 1082, Ref. 2616/220199.