

## **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. 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 (according to the OIV method =12,5 mg/l) =12.6 mg/l for red wines (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

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