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

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 coeffiency 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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