

**OIV-MA-AS315-03 Malvidin diglucoside**

Type IV method

**1. Principle**

Malvidin diglucoside, oxidized by nitric acid, is converted to a substance that, in an ammonium medium, emits a vivid green fluorescence in ultraviolet light.

The intensity of the fluorescence of the compound formed is measured by comparison with the fluorescence of a solution titrated with quinine sulfate whose intensity of fluorescence is standardized with the malvidin diglucoside reference.

Free sulfur dioxide, which attenuates the fluorescence, must previously be combined with excess acetaldehyde.

**2. Qualitative Examination****1. Apparatus**

1. Ultraviolet lamp permitting measurement at 365 nm.

**2.2. Reagents****2.2.1. Acetaldehyde solution**

|                            |      |
|----------------------------|------|
| Crystallizable paraldehyde | 10 g |
|----------------------------|------|

|                   |        |
|-------------------|--------|
| Ethanol 96% (v/v) | 100 mL |
|-------------------|--------|

2.2.2. Hydrochloric acid, 1.0 M.

2.2.3. Sodium nitrate solution, 10 g/L.

2.2.4. Ethanol, 96% (v/v), containing 5% concentrated ammonia solution ( $\rho_{20} = 0.92$  g/mL).

2.2.5. Control wine containing 15 mg of malvidin diglucoside per liter.

2.2.6. Wine containing no malvidin diglucoside.

**2.3. Method**

Into a test tube add:

- 10 mL of wine
- 1.5 mL of acetaldehyde solution

wait 20 minutes.

Into a 20 mL centrifuge tube place:

- 1 mL of wine reacted with acetaldehyde
- 1 drop of hydrochloric acid
- 1 mL sodium nitrate solution

Stir; wait 2 minutes (5 minutes maximum); add:

- 10 mL ammoniacal ethanol

Treat similarly 10 mL of wine containing 15 mg/L malvidin diglucoside (The control wine). Stir. Wait 10 minutes and centrifuge.

Decant the clear liquids from the top into calibrated test tubes. Observe the difference in green fluorescence between the test wine and the control wine under ultraviolet light at 365 nm.

For rose wines, it is possible to increase the sensitivity using:

- 5 mL of wine treated with acetaldehyde (2.3)
- 0.2 mL hydrochloric acid, 1 M (2.2.2)
- 1 mL sodium nitrate solution, 10 g/L (2.2.3)
- 5.8 mL ammoniacal ethanol (2.2.4)

Treat the control wine in a similar manner.

### 2.4. Interpretation

Wines that do not fluoresce, or have a distinctly lower fluorescence, than the control, may be considered to have no malvidin diglucoside. Those whose fluorescence is slightly less than, equal to, or greater than the control should have a quantitative determination.

## 3. Quantitative Determination

### 1. Apparatus

#### 1. Equipment for measuring fluorescence:

- excitation wavelength 365 nm;
- wavelength of fluorescent radiation 490 nm.

#### 2. Optical quartz cell (1 cm path length)

### 3.2. Reagents

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3.2.1. See qualitative examination

3.2.2. 2 mg/L quinine sulfate solution

Prepare a solution containing 10 mg very pure quinine sulfate in 100 mL sulfuric acid, 0.1 M. Dilute 20 mL of this solution to 1 liter with sulfuric acid solution, 0.1 M.

3.3. Procedure

Treat the wine by the method described in *Qualitative examination (2)*, except that the aliquot of acetaldehyde treated wine is each case (red wines and roses) 1 mL.

Place the 2 mg/L solution of quinine sulfate in the cell, adjust the fluorometer to the full range (transmission T, equal to 100%) by adjusting the slit width or the sensitivity.

Replace this tube with the one containing the test wine: this is the T<sub>1</sub> value.

If the percentage of transmission, T<sub>1</sub> is greater than 35, dilute the wine with wine without malvidin diglucoside whose fluorescence must be less than 6% (this should be ascertained by previous testing.)

*Remarks:*

- Salicylic acid (sodium salicylate) added to the wine for stabilization before analysis, causes a spurious fluorescence which can be eliminated by an extraction with ether.
- Spurious fluorescence is caused by the addition of caramel.

3.4. Calculation

A fluorescence intensity of 1, for wine without SO<sub>2</sub>, for the operating conditions above with the exception of the acetaldehyde treatment, corresponds to 0.426 mg malvidin diglucoside per liter of wine.

On the other hand, red and rose wines, containing no malvidin diglucoside, give fluorescence corresponding to a T value of the order of 6%.

The amount of malvidin diglucoside in wine in milligrams per liter is therefore:

$$(T_1 - 6)0,426 \times \frac{11,5}{10} = (T_1 - 6) \times 0,49$$

If the wine is diluted, multiply the result by the dilution factor.

3.5. Expression of the Results

The amount of malvidin diglucoside is expressed in milligrams per liter of wine to the nearest whole number.

### Bibliography

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