# OIV-MA-AS315-05A Hydroxymethylfurfural (HMF)

**Type IV method**

## 1. Principle of the method

Aldehydes derived from furan, the main one being hydroxymethylfurfural, react with barbituric acid and para-toluidine to give a red compound which is determined by colorimetry at 550 nm.

Free sulfurous acid interferes with the determination. When its amount exceeds 10 mg/L, it must be previously eliminated by combining it with acetaldehyde whose excess does not interfere with the determination.

## 2. Colorimetric method

2.1. Apparatus

2.1.1. Spectrophotometer for making measurements between 300 and 700 nm.

2.1.2. Glass cells with optical paths of 1 cm.

2.2. *Reagents*

2.2.1. Barbituric acid solution, 0.5% (*m/v*)

Dissolve 500 mg of barbituric acid in distilled water by heating slightly over a water bath at 100°C. Make up to 100 mL with distilled water. This solution keeps for about a week.

2.2.2. Para-toluidine solution, 10% (*m/v*).

Place 10 g of para-toluidine in a 100 mL volumetric flask; add 50 mL of iso-propanol, $CH_3CH(OH)CH_3$, and 10 mL of glacial acetic acid, $CH_3COOH$ ($\rho_{20}$ = 1.05 g/mL). Make up to 100 mL with iso-propanol. This solution should be renewed daily.

2.2.3. Acetaldehyde (ethanal) solution, 1% (*m/v*).

Prepare just before use.

2.2.4. Hydroxymethylfurfural solution, 1 g/L.

Prepare dilutions of the above solution to containing 5, 10, 20, 30 and 40 mg hydroxymethylfurfural/L. The 1 g/L solution and its dilutions must be freshly prepared.

2.3. Procedure

2.3.1. Preparation of sample

- Free sulfur dioxide less than 10 mg/L:

Perform the analysis on 2 mL of wine or must. If necessary filter the wine or must before analysis.

- Free sulfur dioxide greater than 10 mg/L:

15 mL of the test samples are placed in a 25 mL spherical flask with 2 mL acetaldehyde solution (2.2.3). Stir. Wait 15 minutes. Bring to volume with distilled water. Filter if necessary. Perform the analysis on 2 mL of this solution.

2.3.2. Colorimetric determination

Into each of two 25 mL flasks, *a* and *b*, fitted with ground glass stoppers, place 2 mL of the sample prepared as in 2.3.1. Place in each flask 5 mL of para-toluidine solution (2.2.2); mix. Add 1 mL of distilled water to flask *b* (control) and 1 mL barbituric acid (2.2.1) solution to flask *a*, shake to mix. Transfer the contents of the flasks into spectrophotometer cells with optical paths of 1 cm. Zero the absorbance scale at a wavelength of 550 nm using the contents of flask *b*. Follow the variation in the absorbance of the contents of flask *a*; record the maximum value A, which is reached after 2 to 5 minutes.

Samples with hydroxymethylfurfural concentrations above 30 mg/L must be diluted before the analysis.

2.3.3. Preparation of the calibration curve

Place 2 mL of each of the hydroxymethylfurfural solutions of 5, 10, 20, 30 and 40 mg/L into two sets of 25 mL flasks, a and b, and treat them as described in 2.3.2.

The graph representing the variation of absorbance with the hydroxymethylfurfural concentration in mg/L should be a straight line passing through the origin.

2.4. Expression of results

The hydroxymethylfurfural concentration is obtained by plotting on the calibration curve the absorbance determined on the sample analyzed, taking into account any dilution carried out.

The result is expressed in milligrams per liter (mg/L) to one decimal point.