

## **OIV-MA-AS315-05B Hydroxymethylfurfural (HMF)**

### Type IV method

#### **1. Principle of the method**

Separation through a column by reversed-phase chromatography and determination at 280 nm.

Procedures described below are given as examples.

#### **2. High-performance liquid chromatography**

##### 1. Apparatus

1. High-performance liquid chromatograph equipped with:

- a loop injector, 5 or 10  $\mu\text{L}$
- spectrophotometric detector allowing measurement at 280 nm
- column of octadecyl-bonded silica (e.g. Bondapak  $\text{C}_{18}$ -Corasil, Waters Ass)
- a recorder, preferably an integrator
- Flow rate of mobile phase: 1.5 mL/minute
- Membrane filtration system with a pore diameter of 0.45  $\mu\text{m}$ .

##### 2.2. Reagents

2.2.1. Double distilled water

2.2.2. Methanol, distilled or HPLC quality

2.2.3. Acetic acid ( $d_{20} = 1.05 \text{ g/mL}$ )

2.2.4. Mobile phase: water + methanol + acetic acid previously filtered through a 0.45  $\mu\text{m}$  membrane filter, (40 mL + 9 mL + 1 mL)

The mobile phase must be prepared daily and degassed before using.

2.2.5. Hydroxymethylfurfural reference solution, 25 mg/L ( $m/v$ )

Into a 100 mL volumetric flask, place 25 mg of hydroxymethylfurfural accurately weighed, and bring to volume with methanol. Dilute this solution 1/10 with methanol and filter through a 0.45  $\mu\text{m}$  membrane filter.

If the solution is kept refrigerated in a hermetically sealed brown glass bottle it should

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Hydroxymethylfurfural (HPLC) (Type-IV)

---

keep for two to three months.

### 2.3. Procedure

Inject 5 (or 10)  $\mu\text{L}$  of the sample prepared as described above and 5 (or 10)  $\mu\text{L}$  of hydroxymethylfurfural reference solution into the chromatograph. Record the chromatogram.

The retention time of hydroxymethylfurfural is about six to seven minutes.

### 2.4. Expression of the Results

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