

## **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

Hydroxymethylfurfural (HPLC) (Type-IV)

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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.