

## **OIV-MA-F1-02 Hydroxymethylfurfural (HMF) by High-Performance Liquid Chromatography**

Type IV method

### **1. Principle of the Method**

High-performance liquid chromatography (HPLC)

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

### **2. Reagents**

2.1. Purified water for laboratory use and of quality standard EN ISO 3696

2.2. Methanol, CH<sub>3</sub>OH, distilled or HPLC quality. – CAS Number 67-59-1

2.3. Acetic acid, CH<sub>3</sub>COOH, ( $\rho_{20}$  = 1.05 g/ml). – CAS Number 64-19-7

2.4. Mobile phase: water (2.1) -methanol (2.2)-acetic acid (2.3) previously filtered through a membrane filter (0.45  $\mu$ m), (40:9:1 v/v).

This mobile phase must be prepared daily and degassed before use.

2.5. Reference solution of hydroxymethylfurfural, 25 mg/l (m/v).

Into a 100 ml volumetric flask, place 25 mg of hydroxymethylfurfural, C<sub>6</sub>H<sub>3</sub>O<sub>6</sub>, accurately weighed, and make up to the mark with methanol (2.2). Dilute this solution 1/10 with methanol (2.2) and filter through a membrane filter (0.45  $\mu$ m).

If kept in a hermetically sealed brown glass bottle in a refrigerator, this solution will keep for two to three months.

(The concentration of the reference solution is given for guidance)

### **3. Equipment**

3.1. Apparatus

High-performance liquid chromatograph equipped with:

- a loop injector, 5 or 10  $\mu$ l, (as an example),
- spectrophotometric detector for making measurements at 280 nm,
- column of octadecyl-bonded silica (e.g.: Bondapak C18 — Corasil, Waters Ass.),
- a recorder and, if required, an integrator,

Flow rate of mobile phase: 1.5 ml/minute (as an example).

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3.2. Membrane filtration apparatus, pore diameter 0.45 µm.

### 4. Procedure

#### 1. Preparation of sample

Use the solution obtained by diluting the rectified concentrated must to 40% (m/v) (introduce 200 g of accurately weighed rectified concentrated must into a 500 ml volumetric flask. Make up to the mark with water and homogenise) and filter it through a membrane filter (0.45µm).

#### 4.2. Chromatographic determination

Inject 5 (or 10) µl of the sample prepared as described in paragraph 4.1. and 5 (or 10) µl of the reference hydroxymethylfurfural solution (2.5) into the chromatograph. Record the chromatogram.

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

The volume injected and the sequence are given for guidance. The chromatographic determination can also be done with a calibration curve

### 5. Expression of results

The hydroxymethylfurfural concentration in rectified concentrated musts is expressed in milligrams per kilogram of total sugars.

#### 5.1. Method of calculation

Let the hydroxymethylfurfural concentration in the 40% (m/v) solution of the rectified concentrated must be C mg/l.

The hydroxymethylfurfural concentration in milligrams per kilogram of total sugars is given by:

$$250 \times C/P$$

P = percentage (m/m) concentration of total sugars in the rectified concentrated must.

### 6. Characteristics of the method

Repeatability (r)

- r = 0.5 mg/kg total sugars

Reproducibility (R)

- R = 3.0 mg/kg total sugars