

## **OIV-MA-F1-04 Sucrose by High-Performance Liquid Chromatography**

Type IV method

### **1. Principle**

For testing and determination by high-performance liquid chromatography: the sucrose is separated in a column of alkylamine-bonded silica and detected by refractometry. The result is quantified by reference to an external standard analysed under the same conditions.

*Note:* Authentication of a must or of a wine may be checked by the method using NMR of deuterium described for detecting the enrichment of musts, rectified concentrated musts and wines.

The chromatographic conditions are given for guidance.

### **2. Reagents**

- 2.1. Purified water for laboratory use and of quality EN ISO 3696..
- 2.2. HPLC quality acetonitrile ( $\text{CH}_3\text{CN}$ ) – CAS Number 75-05-8
- 2.3. Sucrose – CAS Number 57-50-1
- 2.4. Mobile phase: acetonitrile-water (80:20 v/v), previously subjected to membrane filtration (0.45  $\mu\text{m}$ ); the composition of the mobile phase is given as an example.  
This mobile phase must be degassed before being used.
- 2.5. Standard solution: 1.2 g/l aqueous sucrose solution. Filter using a 0.45  $\mu\text{m}$  membrane filter. (The concentration of the standard solution is given as an example.)

### **3. Equipment**

1. High-performance liquid chromatograph equipped with:
  - 10  $\mu\text{l}$  loop injector (as an example)
  - a detector: a differential refractometer or an interferometer refractometer
  - an alkylamine-bonded silica column, length 25 cm, internal diameter 4 mm (as an example)
  - a guard column filled with the same phase (as an example)

# COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS

## Sucrose by high-performance liquid chromatography (Type-IV)

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- an arrangement for insulating the guard column and analytical columns or for maintaining their temperature (30 ° C),
- a recorder and, if required, an integrator,
- mobile phase flow rate: 1 ml/min (as an example).

2. Equipment for membrane filtration (0.45 µm).

### 4. Procedure

#### 4.1. Preparation of sample:

Use the solution obtained by diluting the rectified concentrated must to 40 % (m/v) as described in Annex H 'Total acidity', section 5.1., and filtering it using a 0.45 µm membrane filter.

#### 4.2. Chromatographic determination

Inject in turn into the chromatograph 10 µl of the standard solution and 10 µl of the sample prepared as described in 4.1.

Repeat these injections in the same order.

Record the chromatogram.

The retention time of the sucrose is approximately 10 minutes.

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

### 5. Calculations

For the calculation, use the average of two results for the standard solution and the sample.

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

The sucrose concentration in g/kg of the rectified concentrated must is then:

$$2.5 \times C$$

### 6. Expression of results

The sucrose concentration is expressed in grams per kilogram, to one decimal place.

### 7. Characteristics of the method

Repeatability (r)

- $r = 1.1 \text{ g/kg must}$