

## **COEI-2-SUCSAC Grape sugar: Determination of saccharose by HPLC**

### **1. Principle**

The samples diluted or put in solution are analysed by high performance liquid chromatography: Separation on column of grafted silica NH<sub>2</sub> and detection using a differential refractometer.

### **2. Apparatus and analytical conditions (for example)**

#### 2.1. Chromatograph

- Grafted silica column NH<sub>2</sub> (length 20 cm, internal diameter 4 mm granulometry 5 µm)
- A pumping system
- An auto-sampler (maybe)
- Microfritres with porosity 0.45 µm
- Differential refractometry detector

#### **2.** Chromatographic conditions (given as an example)

The water used is deionised and microfiltered.

The acetonitrile is of HPLC quality

The composition of the mobile phase is the following:

- If the column is new: acetonitrile/water (75/25)
- When the fructose - glucose resolution starts to deteriorate, the mobile phase is then a acetonitrile/water 80/20 mixture.

The flow is 1 ml/min.

### **3. Reagents and calibration solutions**

#### 3.1. Preparation of the reference solution

The chemicals used for the reference solution preparation are of "pure for analysis" quality.

The composition of this solution is about 10 g/l for each sugar (fructose, glucose and

INTERNATIONAL OENOLOGICAL CODEX  
Saccharose- Grape sugar- Determination

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saccharose).

The reference solution is prepared every two weeks (maximum) and stored in the refrigerator in the 100 ml graduated flask used for the preparation.