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_2 and detection using a differential refractometer.

2. Apparatus and analytical conditions (for example)

2.1. Chromatograph

- Grafted silica column NH_2 (length 20 cm, internal diameter 4 mm granulometry 5 µm)
- A pumping system
- An auto-sampler (maybe)
- Microfiltres 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

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.