

Determination of WSC by HPLC

1. Determination of fructan, sucrose, glucose and fructose by HPLC

- 1.1 Weigh 1.0000 g of the sample (dried at 60 °C or freeze dried, milled to 1 mm) in a 100 ml graduated flask in two replicates.
- 1.2 Add 70 ml demineralised water (80 °C) and move it slightly at 80 °C in a water bath for 30 min.
- 1.3 After cooling down the flask is filled with water at 20 °C and filtered through a fluted filter S u. S 595 ½.
- 1.4 1 ml of the extract is filled in a vial and analysed by HPLC.
- 1.5 Chromatographic conditions:
 - Column: Rezex RPM Monosaccharide Phenomenex Ltd.
 - Oven temperature: 80 °C
 - Flow: 0,6 ml/min demineralised water
 - RI - Detector Shodex RI-71
 - Injection volume: 20µl
 - Autosampler 460, Oven Controler 480, Pump 420
 - Kontron Instruments
 - Running time: 25 min/sample

2. Interpretation by extern standards (linear regression)

STD1:

each 20 mg Fructan (Orafti – Raftiline HP (storage carbohydrate from chicory))

Sucrose (Fluka)

Glucose (Fluka)

Fructose (Fluka)

diluted in 100 ml demineralised water = 2 %

STD2:

Dissolve each 100 mg of the above mentioned substances in 100 ml demineralised water.