

CARBOHYDRATES ANALYSIS IN BEER BY HPLC

1. Equipment

1.1. Equipment Used for Reagent Preparation

- Balance, capable of an accuracy of 0.0001 g
- Volumetric flasks (typically 100 mL and 2000 mL)

1.2. Equipment Used in Sample Preparation

- Filters, 0.45 μm pore size, equivalent to Millex - HV
- Vials, size suitable to autosampler (polypropylene is acceptable)
- Septa, to fit vials
- Vial caps (septa and vial caps may come as one unit)
- Plastic syringe, 3 mL, with luer-lock end

1.3. HPLC Equipment

- HPLC Solvent Delivery Pump
- HPLC Refractometer detector
- HPLC Autosampler
- Column: GRACE Prevail Carbohydrate ES 5 μs , 250 mm ID:4.6 mm
- In-line solvent degassing unit

1.4. Data Handling Equipment

- Computer integration system capable of controlling and handling results from HPLC. Program must allow extensive editing of peak baseline parameters, beginning and end points, and identification as well as addition and deletion of peaks. *Chromeleon Software*
- Interface Module capable of linking all the components of an HPLC system.

2. Eluents

- Eluent Solution - 65% acetonitrile solution

3. Sample Size - Preparation

- Degas beer samples by vigorous agitation.
- Place a Millex filter on the end of a 5 mL syringe with the plunger out.
- Pour the sample into the syringe and push it through the filter with the plunger, discarding the first and last one millilitre quantities and collecting the middle fraction in an autosampler vial.
- Seal the vial with a septum and vial cap.

4. Calibration curve

4.1. Stock solution

- Add around 2.0000 g of Fructose, 2.0000 g of D-glucose, 2.0000 g maltose monohydrate, 2.0000 g of maltotriose, 1.0000 g of sucrose and 1.0000 g of maltotetraose to a 100 mL volumetric flask.
- Record real weights of each of these compounds, these will be needed when calculating final concentrations.
- Dissolve the contents with deionised water and make up to volume.

4.2. Working standards

- Level 4: Filter your stock standard into a 2ml vial
- Level 3: Pipette 800 uL off the stock standard and 200 uL deionized water Into a 2ml vial
- Level 2: Pipette 500 uL off the stock standard and 500 uL deionized water Into a 2ml vial
- Level 1: Pipette 300 uL off the stock standard and 700 uL deionized water Into a 2ml vial
- Cap them and invert them to mix

4.3. Certified external standard – Customized sugar standard from ERA in Nanopure Deionized Water

Parameter	Value (%w/w)
Fructose	1.00
Glucose	1.00
Sucrose	1.00
Maltose	1.00
Maltotriose	1.00
Maltotetraose	0.500

5. Procedure- work instructions

5.1. Preparing the HPLC for Analysis

HPLC #1: This HPLC is specifically used for Carbohydrate analysis

- Prepare fresh mobile phase
- Once the mobile phase is prepared, perform a dry prime
- To do this go to the display screen on the HPLC and choose *Menu*→*Direct Control*→*Select a duration of 5.00min*→*open C*
- Once the dry prime is finished on the display screen of the HPLC choose *Direct Control*→*Wet Prime*→*Select a flow of 7.5mL/min for duration of 5.0min.*
- Once the wet prime is finished on the display screen of the HPLC choose *Direct Control*→*Purge Injector*→*Select a sample loop volume of 6.0 and yes to compression check.*

The above steps help to ensure that no air locks occur in the column during your sample run.

5.2. Preparing the Column for Analysis

- Open up the software.
- Click the *Run Samples* icon.
- Next select *HPLC #1* and *Carbohydrates* and click *OK*.
- In the flow box enter **0.1** ml of eluent
- Once you click *OK* the HPLC will establish flow to the column. Now go to the **Waters 410 Differential Refractometer** (to the right of the HPLC when looking at it from the front) and

select **2nd function** (Blue Button) then Button **#9(purge)**. Purge the detector for a minimum of 30 minutes. After the purging is complete deselect the purge option by selecting **2nd function** then **Button #9 (purge)** again.

- Purging of the column is done at a low flow rate
- In order to analyze your samples flow to the HPLC column now has to be increased.

5.3. Running the sequence

- While the instrument is equilibrating, enter the information for the sequence you wish to run.
- Start Acquisition Mode on the Software for a minimum of 30 min. The system could take longer to equilibrate. Observe baseline and do not run the sequence until baseline has reach an stable stage
- Run sequence

5.4. Extracting Results from the Software

- Review each Chromatogram to ensure every peak of interest has been properly integrated by the software
- Review Calibration curve. Coefficient of determination should be at least 99.5% in order to submit results for that particular sequence

6. Calculations and Expression of Results

- Use the amount weighed to calculate the concentration of the stock solution and the working standards.
- Enter the concentrations in the Amount table of the QNT editor. The software will calculate the amounts of the unknown samples automatically.
- Print the report and submit results

7. References

- American Society of Brewing Chemists, Methods of Analysis, 14B