HPLC Analysis of Sugars in Alcoholic Beverages

Scope and Application

The objective of this method is to determine fructose, glucose and sucrose in alcoholic beverages. TTB Ruling 2004-1 requires that all Alcohol Facts Labels include a statement of average analysis for calories, fat, carbohydrate and protein. ***S***ugar is used in the calculation of the caloric content of wines***, flavored malt beverages, and some distilled spirits if labelled.***

This method utilizes high performance liquid chromatography with a strong cation exchange column to separate mono- and some disaccharide sugars.

Regulatory Tolerances

27 CFR 4.21(a) and 27 CFR 5.23(a)(3) regulate sugar addition in wines and vodka. Statements of sugar (late harvest wines) or degree brix should not vary by 10% over or under the amount stated on the label. For wines with a stated level of 1% or less sugar, the actual value must be between zero and 1%. Products with nutritional labeling (e.g., coolers) should not vary by 10% over or under the amount stated on the label. The use of sugar in vodka is authorized to a maximum of 2 grams per liter.

Levels and Limitations

1. Most wines need to be diluted x 10 with ***deionized*** water. ***All samples should be diluted to at least x 2 with DI water to reduce any matrix interferences.*** If the sample has a known amount of sugars (check nutrition label), the sample needs to be diluted such that it falls within the range of the standard curve.
2. Column life is limited to approximately 600 injections. Peaks tend to develop shoulders as the column ages; therefore, the analyst needs to routinely monitor column performance by running standards at the start and end of each run.
3. If necessary, samples may need to be calculated with standards run during the middle or end part of the sequence. For example, if a change in retention time occurs during the run or if after the sample is injected the analyst realizes that re-dilution is needed, the new dilution may be added to the run, and a matching (+/- 10%) standard injected prior or after the sample run may be used to calculate the unknown sample. Alternatively, the analyst can also opt to rerun a sample after a run is completed, using the same method file together with a standard or LCS injected as an unknown to show that the run parameters have not changed.
4. If there is not enough equilibration time between samples, possible carry over from the previous sample may occur. Glycerol elutes at around 12 minutes close to the ethyl alcohol peak and should not interfere with this analysis.

Limits for Each Analyte *(Sucrose, Glucose, and Fructose):*

Detection Limit (g/100 mL)	Quantitation Limit (g/ 100mL)	Linear Range (g/ 100mL)	*Calibration Range (g/100mL)*	Interferences
0.005	0.02	0.02-0.5***0***	*0.05 – 0.50*	Possible matrix carryover. See #4.

Equipment

Instrumentation:

HPLC: Waters 2695 Alliance Liquid Chromatograph with column heater or equivalent

Column: Waters Sugar Pak1 Column, 6.5 X 300 mm

Guard Column: Amino 5µ and C18 5µ

Mobile Phase: Deionized water

Flow: 0.6 m***L***/min
CAUTION: refer to step 1 in Procedure Notes below

Injection: 20 µL

Temperature: 85ºC

Detector: Waters 2410 Refractive Index Detector or equivalent

Run Time: 20 min

Glassware and Supplies:

Class A volumetric pipets (or ***auto*** pipettor), volumetric flasks, and other assorted laboratory glassware.

Reagents, Sample Preparation and Handling

Reagents:

Deionized (DI) water
Sucrose,**≥** 99% purity
Glucose, **≥**99% purity
Fructose, ***≥99% purity***
Ethyl Alcohol, 200 Proof, ***diluted to 20% with DI water***

Reagent Preparation:

20% Ethyl Alcohol Solution:

For example:

Transfer 20 mL Ethyl Alcohol into a beaker, add 80 mL DI water and mix thoroughly.

Stock and Working Standard Preparation:

Prepare **1%** (1 g/100 mL) **Mixed Stock Sugar Standards*:***
For example:

1. Weigh 1.00 (±0.004) g of each of the sugars into a 100 mL volumetric flask.

   Fill to volume in 20% ethyl alcohol-water. S***tandard is s***table for 1 month ***when*** refrigerated.

Prepare Working Calibrants as follows: Prepare fresh daily.

1. Into separate 10 mL volumetric flasks, transfer 5 and 2 mL of the stock standard and dilute to volume with DI water. This will correspond to a working dilution of 0.5 and 0.2 g/100 mL.
2. From the 0.5 g/100 mL working standard, transfer 1 mL into a third 10 mL volumetric flask and dilute to volume with ***DI*** water. This will correspond to 0.05 g/100mL.

Sample Preparation

Generally, wine samples need to be diluted x 10 with ***DI*** water. ***All samples should be diluted to at least x 2 with DI water to reduce any matrix interferences. If applicable, use sugar label claims to determine the dilution factor needed.*** Degas carbonated ***samples*** by sonication or other appropriate degassing method before analysis.

Two Laboratory Control Sample***s*** (LCS) ***are*** run with each batch to monitor performance. The LCS can either be a sample with known sugar content or a sample spiked with known amount of sugar. Dilute appropriately.

Procedure

Note : If the column has been in storage for more than a week, an overnight flush at 0.1 mL/min with ***DI*** water is recommended to ***rehydrate*** the column.

1. Set the mobile phase flow to 0.2 mL/min. Wait until column temperature reaches 85ºC then gradually increase flow in 0.2 m***L*** increments until you reach 0.6 mL/min. Be sure to wait for column pressure to stabilize before increasing the flow rate.
2. Inject 20 µL of ***DI*** water blank and 3 working standards to generate a calibration curve using the system software. (Blank is not part of the calibration curve).
3. Inject 20 µL samples and controls (intermediate standards ***and*** LCS***s***). Determine sugar concentration utilizing the calibration curve and system software.
4. Run blanks and standards at the end of each run to ensure system is operating as expected.

Quality Control

Run the 3 working standards. The ***DI*** water blank will serve to indicate the absence of any interference from the mobile phase, column, and other sources. Determine the linearity of the curve.

Analysis should only proceed if the correlation coefficient is greater than 0.99. Run standard as unknown after at least every 10 samples and insert blank to check for carryover after every suspected high sample.

Run ***two*** laboratory control samples (LCS) ***and compare against established quality control limits.***

Run an intermediate standard***, which must*** be within ± 10% of the e***xpected*** value.

Any samples above the range of the standard curve need to be diluted and retested. Conversely, if the sample is too dilute, re-inject with a less diluted sample ***or report as ND or BQL as appropriate***.
If results for LCS or standard as unknown are out of the expected ranges, confirm that instrument is operating correctly (syringe blockage, mobile phase, volume in vial etc). If needed, prepare fresh LCS/std and reinject.

<u>Sources of Measurement Uncertainty</u>
-Sample dilution
-Standard preparation
-Instrument performance
-Inadequate column equilibration
-Column condition

Calculation

Use the operating system software to generate a linear equation using peak height (or area). Sugars are confirmed by comparing retention times with those of standards. ***S***ugars are

calculated in grams per 100 mL. ***Reporting sugars by serving size may be necessary for label comparison.***

If the sample had been diluted, multiply the result by the dilution factor to obtain the concentration in the original sample.

Reporting Results

Report sugars to ***two*** decimal place***s***, i.e. ***xx.XX, in units based on established serving sizes:***

Wines: To convert g/100 mL sugar to ***g/***5 fl oz; use 148 mL for the calculation.
Distilled Spirits: To convert g/100 mL sugar to g/1.5 fl oz; use 44 mL for the calculation.
Beer: ***To c***onvert g/100 mL sugar to g/***12 fl oz; use*** 355 mL ***for the calculation***.

Or as required by proficiency test guidelines (e.g., in terms of Glucose + Fructose g/100 mL)

Or as appropriate (e.g., in terms of g/100 mL)

Safety Notes

Normal laboratory safety protocol should be followed.

References

TTB Ruling 2004-1
27 Code of Federal Regulations

Required Training, Certification and Re-certification

1. Initial in-house training by certified chemist in standard preparation, HPLC operation.
2. Initial certification is achieved by running ***at least 7 LCSs*** with results ***within established limits***.
3. Annual proficiency testing.

Revision History:

Rev. 3 – Added criteria for acceptance of LCS and intermediate check (8/21/2009)

Rev. 4 – Changed reporting units to 2 decimals (xx.XX); removed requirement for 18 MΩ water; added “Example is Fructose” to calculation; removed “s” from standard in QC section for running a check sample not samples. – 3/22/2012

Rev. 5 – Updated language in the Scope to include all alcoholic beverages. Added suggestion to dilute all samples. Updated the limits table, and added the calibration range. Updated the Reagent preparation section. Updated the Reporting Results section. Added requirement to run at least 7 LCS for initial certification. Changed name from Residual Sugars to Sugars. Changed all references of MQ to DI water.