



DEPARTMENT OF THE TREASURY
ALCOHOL AND TOBACCO TAX AND TRADE BUREAU
SCIENTIFIC SERVICES DIVISION
WASHINGTON, DC 20220

OFFICIAL METHOD — SSD:TM:301

Residual Sugars in Alcohol Beverages by HPLC

Scope and Application

This method is used to determine the residual sugar content of alcohol beverages. This result can then be used as a basis for the calculation of their carbohydrate and calorie content. This method applies to flavored wines, cooking wines, specialty products (coolers), and distilled spirits.

This method is based on the selectivity of mono- and some disaccharide sugars to separate when passed through an ion-exchange column containing a strong cation form.

Regulatory Tolerances

27 CFR 4.21(a) and 27 CFR 5.23(a)(3) regulate sugar additions in wines and vodka. Additionally, statements indicating residual sugar or degree brix should not vary by more than 10% above or below the stated value on the label. Wines labeled as having 1% (1.428 g per 5 fl oz), or less, residual sugars must have an actual value between zero and 1%. Products with nutritional labeling (coolers) should not vary by more than 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.

TTB Ruling 2004–1 requires that all Alcohol Fact Labels include a statement of average analysis for calories, fat, carbohydrate, and protein.

Equipment

Instrumentation:

HPLC:	WATERS 2695 Alliance Liquid Chromatograph with column heater capable of reaching 85°C and Empower software, or equivalent system.
Column:	WATERS Sugar Pak1, 6.5 x 300 mm.
Guard Column:	Amino 5μ and C18 5μ.
Mobile Phase:	Deionized 18.2 megaO water, degassed with He if on-board degassing is not available.

Flow: Set the mobile phase flow to 0.2 ml/min. Wait until column temperature reaches 85°C and then gradually increase the mobile phase flow to 0.6 ml/min.

Temperature: 85°C

Detector: Waters 2410 Refractive Index Detector, or equivalent.

Injection Volume: 20 µL

Run Time: 20 min.

Glassware and Supplies:

Class A volumetric pipets, flasks, and other assorted laboratory glassware.

Reagents, Sample Preparation and Handling

Regents:

- Deionized water, 18 megaO or better;
- Sucrose, 99.9% purity, Fisher Scientific;
- Dextrose, 99.9% purity, Fisher Scientific;
- Fructose, Reagent Grade, Fisher Scientific; and
- Ethyl Alcohol, 200 Proof, Gold Shield Chemical Co., Hayward, CA.

Reagent Preparation:

1. Prepare a *1% Mixed Stock Sugar Standard* by weighing 1.00 (±0.04) g of each of the sugars into a 100 mL volumetric flask. Q.S. to volume in 20% ethyl alcohol-water. Prepare fresh every week. Store refrigerated.
2. Prepare Working Standards:
 - o *Working Standard 1 (0.5%)*: Transfer 5 mL of the stock standard and dilute to volume with MQ water. Prepare fresh daily.
 - o *Working Standard 2 (0.2%)*: Transfer 2 mL of the stock standard and dilute to volume with MQ water. Prepare fresh daily.
 - o *Working Standard 3 (0.05%)*: From the 0.5 percent working standard, transfer 1 mL into a third 10 mL volumetric flask and dilute to volume with MQ water. This will correspond to 0.05 percent. Prepare fresh daily.

Sample Preparation:

- For most wines and distilled spirits, no sample preparation is needed. Wines are diluted 1–10 with water and injected directly.
- Carbonated samples should be degassed by sonication. If sample contains residue, filter through a 0.45µ membrane before diluting.
- Reagent blank uses 5 mL of deionized water in lieu of sample.

Procedures

1. Inject 20 µl of blank and 3 standards to generate a calibration curve using the system software.
2. Inject 20µl samples and controls (intermediate standards as unknowns). Determine sugar concentration utilizing the calibration curve and system software.
3. Run blanks and standards at the end of each run to ensure system is operating as expected.

Quality Control

Intermediate control standards should be within 5% of expected values. Curve linearity correlation coefficient for all 3 analytes should be >0.99.

Performance Characteristics and Acceptance Limits

A water blank is run to check baseline stability. Three concentration levels of sugar standards are run at the beginning of the analysis, with intermediate concentrations interspersed as unknowns throughout the run.

Calculations

HPLC is operated in external standard mode, and calculations are performed by integration of peak heights (or area) using the standard curve generated. Identification of sugars is confirmed by comparing the retention times of the standards with those in the samples. With this column, higher molecular weight sugars elute first.

- For wines, convert g/100 mL sugar to g/5 fl oz:

$$\text{g/100 mL sugar} \times 148 \text{ mL/(5 fl. oz.)} = \text{g/5 fl. oz.}$$
- For distilled spirits, convert g/100 ml sugar to g/1.5 fl oz:

$$\text{g/100 mL sugar} \times 44 \text{ mL/(1.5 fl. oz.)} = \text{g/1.5 fl. oz.}$$

Reporting Results

Report sugars or residual sugars in g/5 fl.oz to one decimal place, i.e. XX.X g sugars/ 5 fl. oz for wines or XX.X g sugars/1.5 fl.oz for distilled spirits.

Safety Notes

- Normal laboratory safety protocol should be followed.
- The column used in this analysis is set at a high temperature, use caution when handling.

References

- TTB Ruling 2004–1.
- ATF Ruling 80–3.