Residual Sugars and Erythritol in Alcohol Beverages by UPLC-RI

Scope and Application

This method determines erythritol, fructose, glucose, sucrose, maltose, and lactose in wines, malt beverages, and distilled spirits. Many alcohol beverages contain varying amounts of sugars to enhance flavor. Finished wine contains naturally occurring fructose, glucose, and small amounts of sucrose which adds to its flavor and calorie profile. TTB Rulings 2013-2 and 2020-1 requires that Alcohol Facts Labels include a statement of average analysis for calories, fat, carbohydrate, and protein. The total amount of sugar present in wine, wine cooler or other sugar containing alcohol beverage is used in the calculation of the caloric content of the product when calories are labelled. The wine industry may label a product to indicate sweetness of a wine in terms of degrees brix for informational purposes, particularly in late harvest or dessert style wines. Sugar is sometimes added to vodka as a flavoring; testing for specified limits for sugar in vodka relates to the standard of identity for vodka. Although the US has no regulated sweetness labeling terms, the European Union (EU) does define terms for sparkling wines based on sugar content and for still wines based on sugar content and total acidity.

Regulatory Tolerances:

The addition of sugar in vodka is regulated under 27CFR5.142(b)(1) and 27CFR5.155(c)(3) to a maximum of 2 grams per liter.

Numerical sugar content claims should be made in accordance with the guidance set forth in TTB Procedure 2020-1 with regard to carbohydrate content statements. The procedure states that carbohydrate statements will be considered acceptable as long as the carbohydrate content is “within a reasonable range below the labeled or advertised amount (within good manufacturing practice limitations) but must not be more than 20% above the labeled or advertised amount.” Per laboratory policy, a “reasonable amount below” has been established at -20% or one gram, whichever is larger. So if the sugar is stated as 4 grams/serving, they are compliant if tested from 3.0 grams to 4.8 grams/serving.

The procedure also specifies that the number of grams (g) of sugar in a serving must be expressed to the nearest tenth of a gram, except that if a serving contains less than 1 gram, the statement "Contains less than 1 gram (g)" or "less than 1 gram (g)" may be used as an alternative. If the serving contains less than 0.5 g of sugar, the content may be expressed as zero (or 0) grams (g).

TTB Procedure 2020-1 is aligned with FDA’s regulations regarding the addition of sugar in foods under 21CFR101.9(g)(5) and (6), which also allows for a maximum tolerance of 20% above the declared amount and a reasonable deficiency within current good manufacturing practices.

Wines from the European Union (EU) are tested against the EU definitions.
Commission Regulation (EC) No 607/2009 of 14 July 2009, Annex XIV, page 72:
(A digital copy can be found in the ISO > External Documents folder on the All_SSD drive)

Part A for sparkling wines:

Terms for sparkling wines	Conditions for use – sugar content in g/L
Brut nature	<3
Extra brut	Between 0 and 6
Brut	<12
Extra dry	Between 12 and 17
Dry	Between 17 and 32
Medium dry	Between 32 and 50
Sweet	>50

Part B for still wines:

Terms	Conditions for use
Dry	If its sugar content does not exceed: —4 grams per litre, or —9 grams per litre, provided that the total acidity expressed as grams of tartaric acid per litre is not more than 2 grams below the residual sugar content.
Medium dry	If its sugar content exceeds the maximum set at above but not exceeds: —12 grams per litre, or —18 grams per litre, provided that the total acidity expressed as grams of tartaric acid per litre is not more than 10 grams below the residual sugar content.
Medium or medium sweet	If its sugar content is higher than the maximum set at above but not more than 45 grams per litre.
Sweet	If its sugar content is of at least 45 grams per litre.

Article 58 (page 18) specifies:

- Sugar content is expressed in terms of fructose and glucose (including any sucrose).
- Without prejudice to the conditions of use described in Part A of Annex XIV, the sugar content may not differ by more than 3 grams per litre from what appears on the product label.

Levels and Limitations

Injection	Detection Limit	Quantitation Limit	Linear Range	Interferences
10 µL	0.002 g/100 mL	0.01 g/100 mL	0.01 - 0.4 g/100 mL	Matrix, high sugars
5 µL*	0.01 g/100 mL	0.03 g/100 mL	0.03 - 0.4 g/100 mL	Matrix, high sugars

*For samples with suspected very high sugar levels for example fruit cider with added sugar, analyst has the option to use 5 µL injects after appropriate dilution with mobile phase. In this case, the entire run (standards and samples) must be performed using 5 µL injections.

1. All samples must be diluted at least x 5 with mobile phase for optimum chromatography resolution.

2. If the sample has known amounts of sugar (check label claim) dilute appropriately with mobile phase such that the sugar falls within the range of the calibration curve.

3. Mobile phase blank injections may be necessary when sample carryover is suspected.

Supplemental Documents

SSD:QPD:3100 Laboratory Quality Control (Formerly SSD:QPD:310)
WG:SSD:1040:004 Beer and Wine Degassing Procedure (Formerly SSD:WG:115)
Form:SSD:316:001 Daily Log for SSD:TM:316 Residual Sugars in Beverage Products (Formerly NLC:Form:316-1)
Form:SSD:316:002 Performance Checks for SSD:TM:316 (Formerly NLC:Form:316-2)

Equipment

Analytical balance Mettler Toledo AG 204 Delta Range, or equivalent
Microcentrifuge

Instrumentation and Run Conditions:

UPLC:	Waters UPLC Acquity H Class or equivalent
Detector:	Acquity UPLC RI Detector or equivalent
Autosampler:	Acquity Sample Manager or equivalent
Injection Volume:	10 µL
Temperature Control Compartment:	Acquity CH-A or equivalent
Column Temperature:	35ºC
Quaternary Pump:	Acquity Quaternary Solvent Manager or equivalent
Flow Rate:	0.15 mL/min
Weak Needle Wash	75-25 Acetonitrile - water
Strong Needle Wash	90-10 Water acetonitrile
Mobile Phase:	76-24 Acetonitrile –water + 0.4% TEA
Gradient:	Isocratic run conditions
Run Time:	14.00 minutes
Degasser:	Acquity H Class series degasser, G1322A, or equivalent
Software:	Empower 3 v.7.20.00.00 Base Package or equivalent
Guard and Column:	Acquity UPLC BEH Amide 1.7 µ or equivalent Waters Acquity 1.7µ BEH Amide 2.1x150mm or equivalent

Glassware and Supplies:

1.5 – 2 mL microcentrifuge tubes
Disposable transfer pipettes
2 mL autosampler vials and caps
Class A volumetric flasks, 100 mL or as needed
Graduated cylinders, 50 mL 100 mL and 1 L, or as needed
Autopipettor(s) capable of delivering 50 µl to 1000 µL volume
Autopipettor tips to fit Autopipettor(s) used above.
Magnetic stir-bars, 1 inch or less,

Reagent and Sample Preparation and Handling

Reagents: (Specific vendors are listed for convenience. Equivalent products may be used.)

Deionized (DI) water
Acetonitrile, Optima (CAS 75-05-8), Fisher
Ethanol 200°Proof (CAS 64-17-5), Fisher
Triethylamine (TEA) (CAS 121-44-8, >99%), Sigma

Calibration Standards: (Specific vendors are listed for convenience. Equivalent products may be used). For routine testing of samples, only fructose, glucose and sucrose standards are used.

Primary Stock Standard: Use of certified reference standard is recommended:
Erythritol (CAS 149-32—6, ≥99%), Sigma-Aldrich
Fructose (CAS 57-48-7, ≥99%), Sigma-Aldrich
Glucose (CAS 50-99-7, ≥99.5%) Sigma-Aldrich
Lactose (CAS 5989-81-1, ≥99%) Sigma-Aldrich
Maltose (CAS 6363-53-7, ≥98%) Sigma-Aldrich
Sucrose (CAS 57-50-1, ≥99.7%) Sigma-Aldrich

Secondary stock standards which are not certified as reference may be used in preparing the secondary check standard solution after establishing suitability against certified standards.

Preparation of Solutions:

I. Mobile phase and diluent preparation (Stable for 1 month)

For 200 mL:

1. Transfer 152 mL acetonitrile and 48 mL DI water into an UPLC reservoir containing a stir-bar. Mix.
2. Add 0.8 mL TEA, mix for approximately 30 sec.
3. Depending on the number of samples being tested, reserve a portion (10-20 mL) of the mobile phase to dilute standards and samples (approx. 1 mL/vial).

II. Weak needle wash-specific to Waters instrumentation (Stable for 6 months)

Transfer 750 mL acetonitrile into UPLC reservoir containing a stir-bar.
Add 250 mL DI water and mix. Place on UPLC instrument.

III. Strong Needle Wash/Seal Wash -specific to Waters instrumentation (Stable for 6 months)

Transfer 100 mL acetonitrile into UPLC reservoir containing a stir-bar. Add 900 mL DI water and mix.
Place on UPLC instrument.

Preparation of Calibration and Working Standards:

1. Primary and Secondary Calibration Stock Mix Standard 1 g/100 mL (1%) (Stable for 6 months)

Note: For routine testing fructose, glucose and sucrose standards are used.

For example weigh 1 g of each standard into a 100 mL volumetric flask. Add 20 mL DI water, 20 mL ethanol and 20 mL acetonitrile, mix. Bring to volume with DI water.

2. Working Standards (stable for 2 days when refrigerated)

The following guidance is provided when using micropipettes for dilution into injection vials. Alternatively, working standards may be prepared in appropriate sized volumetric flasks.
Diluent is mobile phase (stable for 1 month).

Level 3 - 0.3 g/100 mL (%): Transfer 300 µL stock standard and 700 µL diluent into vial. Cap and mix.

Level 2 - 0.1 g/100 mL (%): Transfer 100 µL stock standard and 900 µL diluent into vial. Cap and mix.

Level 1 - 0.03 g/100 mL (%): Transfer 100 µL from Level 3 and 900 µL diluent into vial. Cap and mix.

Check standard: For example for a 0.2 g/100 mL (%) concentration, transfer 200 µL from secondary 1% stock standard into a vial. Add 800 µL diluent, cap, and mix.

Preparation of Samples and Laboratory Control Sample (LCS):

Degas carbonated samples prior to analysis using established procedures

1. Dilute samples 5x (or more) prior to injection. For example, transfer 200 µL sample into a microcentrifuge tube and add 800 µL diluent, cap, and mix for a 5x dilution. Additional dilutions may be necessary depending on the sugar content of the sample. Diluted samples are stable for 2 days when refrigerated.

2. Centrifuge all samples, including LCS, for 5 minutes @ 14,800 rpm and transfer supernatant to autosampler vials prior to injection.

Procedures

The following is provided as a guideline:

1. Equilibrate the column in mobile phase for a minimum 1 hour prior to beginning injections.

2. Inject a blank mobile phase to check baseline.

3. Optional-Inject a standard to confirm operating conditions are suitable.
Once conditions are deemed optimal for analysis, inject calibrants and unknown samples per laboratory protocol.

Quality Control

1. Run a minimum of one blank prior to beginning the sequence to check for potential interference and confirm good baseline.

2. R^2 should be >0.99 and calibrant residuals (% deviation) should be within +/- 10% of expected. For calibration failure, prepare fresh calibrants and re-run the sequence. If problem continues, stop run and consult with POC.

3. A laboratory control sample (LCS) is diluted in duplicate and injected. Results must be within the specified tolerances established during LCS characterization.
In case of LCS failure: Troubleshoot to investigate cause (for example dilution error, instrument failure, system equilibration). Once resolved, if LCS passes accuracy, proceed with run. Consult with POC if problems continue. Refer to SSD:QPD:3100 for CAR initiation if necessary.

4. An intermediate check standard is run after every 5-6 samples; for example a 0.2% mix sugar standard. Results must be within +/- 10% of expected. If check standard is out of tolerance, refer to troubleshooting above in 2 and 3 and retest check standard. Report data between acceptable check standard results. If problem persists, stop the run and initiate CAR see SSD:QPD:3100.

5. A blank may be run after a matrix interference is suspected.

6. LCS bottles can be used for 1 month when capped and stored at room temperature.

Sources of Measurement Uncertainty

1. Dilution errors using micropipettes.
2. Incorrect mobile phase preparation.
3. Diluent used for samples and calibration standard do not match the mobile phase preparation.
4. Weighing error.
5. Samples with high sucrose (or other sugar) may require further dilution and reinjection to avoid overloading the detector.
6. May need to inject blanks between samples to avoid possible coelution or peak inhibition.

Calculations

Peak area is used to determine calibration curves. Use software to generate calibration curves and generate results. Multiply the result by the dilution factor to obtain the concentration of the original sample.

The standard serving sizes vs type of product and alcohol content are provided below:

Serving Size	Alcohol Percent by Volume		
	Wine	Distilled Spirits	Malt Beverages
1.5 fl oz (44 ml), or 50 ml for 50 ml containers of distilled spirits		Above 24%	Above 24%
2.5 fl oz (74 ml)	Above 16 to 24%	Above 16 to 24%	Above 16 to 24%
5 fl oz (148 ml)	7 to 16%	Above 7 to 16%	Above 7 to16%
12 fl oz (355 ml)		Not more than 7%	Not more than 7%

Ref: TTB Ruling 2013-2

To report sugars per serving size:
Sugars (g/serving) = sugars (g/100 mL) x serving volume (mL)/100 mL

To report erythritol per serving size:
Erythritol (g/serving) = Erythritol (g/100 mL) x serving volume (mL)/100 mL

For example:
Wines: To convert g/100 mL sugar (result from the LC) to g/5 fl oz; use 148 mL for the volume calculation to give g/5 fl.oz.

To report sugars in Brix or % wt/wt:
Brix or % wt/wt = total sugars (g/100 mL) / density of beverage (g/mL)

Reporting Results

Report each individual analyte and the total sugars (sum of all the analytes except erythritol) to the nearest X.xx in the labelled units or as required by proficiency test guidelines.

Report erythritol to the nearest X.xx in the labelled units or per serving volume

Safety Notes

1. Use protective personal gear when handling acetonitrile and TEA.
2. Dispose of mobile phase, samples, and standards per laboratory guidelines.
3. Dispose LC waste per laboratory guidelines.

References

1. Fountain, K. J., Hudalla, C., McCabe, D., & Morrison, D. (2014). UPLC-MS analysis of carbohydrates. Waters Application Note.
2. TTB Procedure 2020-1
3. TTB Ruling 2020-1
4. TTB Ruling 2013-2
5. 27CFR5.142(b)(1)
6. 27CFR5.155(c)(3)
7. 21CFR101.9(g)(5) and (6)
8. Commission Regulation (EC) No 607/2009 of 14 July 2009

Required Training, Certification, and Re-certification

1. Receive in house UPLC training.
2. Initial certification is achieved by preparing and analyzing 7 LCS with results in agreement with the mean and standard deviation acceptance criteria.
3. Periodically, chemists are retested for competency (e.g. every 5 years) and/or given proficiency test.

Revision History

Rev. 1 – initial revision

Rev. 2 – added erythritol as separate line to calculation section

Rev. 3 – Updated CFR Reference

Rev. 4 – Updated expiration dates for LCS bottle, mobile phase, and sample diluent. Removed references to CL.

Rev. 5 – Added the centrifugation of all samples to the sample preparation and updated references.

Rev. 6 – Changes to the Quality Control section. Changed document ids to the new document id structure. Corrected Level 1 Preparation of Calibration and Working Standards.

Rev. 7 – Removed the requirement for a blank injection at the end of the sequence. Updated Reference section.