High Fructose Corn Syrup

DEFINITION

High Fructose Corn Syrup is a clear aqueous solution of saccharides prepared from high-dextrose-equivalent corn starch hydrolysate by the partial enzymatic conversion of dextrose to fructose, using an insoluble glucose isomerase enzyme preparation that complies with 21 CFR 184.1372. It is available in two types, 42% and 55%, based on fructose as a proportion of total saccharides. High Fructose Corn Syrup 42% contains NLT 97.0% of total saccharides, expressed as a percentage of total solids, of which NLT 92.0% consists of monosaccharides, including 41.0%–50.0% of fructose, and NMT 8.0% consists of other saccharides. High Fructose Corn Syrup 55% contains NLT 95.0% of total saccharides, expressed as a percentage of total solids, of which NLT 94.0% consists of monosaccharides, including 54.0%–60.0% of fructose, and NMT 6.0% consists of other saccharides.

IDENTIFICATION

- **A.**

Analysis: Add a few drops of a solution (1 in 10) of Syrup to 5 mL of hot, alkaline cupric tartrate TS.

Acceptance criteria: A copious, red precipitate of cuprous oxide is formed (distinction from sucrose).

- **B.** It meets the requirements of the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Water

Standard solution: Prepare a solution in water, which contains a total of 10% (w/v) saccharide solids, using USP Dextrose RS, USP Fructose RS, and USP Maltose Monohydrate RS, in proportions that approximate, on the *Total Solids* basis, the composition of High Fructose Corn Syrup to be analyzed (see *Table 1*).

Table 1

Component	High Fructose Corn Syrup 42%	High Fructose Corn Syrup 55%
USP Fructose RS	4.2%	5.5%
USP Dextrose RS	5.0%	4.0%
USP Maltose Monohydrate RS	0.8%	0.5%

Sample solution: Dilute a known weight of Syrup, determined from the result of the test for *Total Solids*, with water to have 10% (w/v) solids.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 7.8-mm × 30-cm; packing L19

Temperatures

Detector: 45°

Column: 85°

Flow rate: 0.6 mL/min

Injection volume: 10 µL

Run time: 20 min

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for maltose, dextrose, and fructose are about 0.83, 1.00, and 1.32, respectively.]

Suitability requirements

Resolution: NLT 1.5 between maltose and dextrose, and NLT 1.5 between dextrose and fructose

Relative standard deviation: NMT 1.0% for the fructose peak

Analysis

Samples: *Standard solution* and *Sample solution*

Inject a volume (about 10 µL) of the *Sample solution*, and measure all the peak areas. The elution pattern includes the higher degree of polymerized saccharides (DP4+), followed by tri-saccharides (DP3), maltose, dextrose, and fructose. The higher-molecular-weight polysaccharides containing more than 4 D-glucopyranosyl units can be integrated into one peak represented by the peak of DP4+.

[NOTE—The relative retention times for higher degrees of polymerized saccharides (DP4+), tri-saccharides (DP3), maltose, dextrose, and fructose are about 0.66, 0.74, 0.82, 1.00, and 1.32, respectively, from the *Sample solution*.]

Calculate the percentage of monosaccharides, P_{MS}, expressed in terms of fructose (P_F) and dextrose (P_D), in the total solid portion of Syrup taken:

$$P_{MS} = P_F + P_D$$

$$P_F = (r_{FU}/r_{FS}) \times [C_{FS} \times V_U/(W_U \times 0.01 \times P_{Solid})] \times 100$$

$$P_D = (r_{DU}/r_{DS}) \times [C_{DS} \times V_U/(W_U \times 0.01 \times P_{Solid})] \times 100$$

r_{FU} = peak area of fructose from the *Sample solution*
r_{FS} = peak area of fructose from the *Standard solution*
C_{FS} = concentration of USP Fructose RS in the *Standard solution* (mg/mL)
V_U = volume of the *Sample solution* (mL)
W_U = weight of Syrup taken to prepare the *Sample solution* (mg)
P_{Solid} = percentage of total solids in the Syrup as determined in the test for *Total Solids*
r_{DU} = peak area of dextrose from the *Sample solution*
r_{DS} = peak area of dextrose from the *Standard solution*
C_{DS} = concentration of USP Dextrose RS in the *Standard solution* (mg/mL)

Calculate the percentage of other saccharides, P_{OS}, expressed in terms of maltose (P_{DP2}), tri-saccharides (DP3) (P_{DP3}), and higher degree of polymerized saccharides (DP4+) (P_{DP4+}), in the total solid portion of Syrup taken:

$$P_{OS} = P_{DP2} + P_{DP3} + P_{DP4+} = [(r_{U1} + r_{U2} + r_{U3})/r_S] \times [C_S \times V_U/(W_U \times 0.01 \times P_{Solid})] \times 100$$

r_{U1} = peak area of maltose from the *Sample solution*
r_{U2} = peak area of tri-saccharides (DP3) from the *Sample solution*
r_{U3} = peak area of higher degree of polymerized saccharides (DP4+) from the *Sample solution*
r_S = peak area of maltose from the *Standard solution*
C_S = concentration of USP Maltose Monohydrate RS in the *Standard solution* (mg/mL)
V_U = volume of the *Sample solution* (mL)
W_U = weight of Syrup taken to prepare the *Sample solution* (mg)
P_{Solid} = percentage of total solids in the Syrup as determined in the test for *Total Solids*

Calculate the percentage of total saccharides, P_{TS}, expressed as a percentage of total solids:

$$P_{TS} = P_{MS} + P_{OS}$$

Calculate the percentage of monosaccharides in total saccharides:

$$\text{Result} = (P_{MS}/P_{TS}) \times 100$$

Calculate the percentage of fructose in total saccharides:

$$\text{Result} = (P_{F}/P_{TS}) \times 100$$

Calculate the percentage of other saccharides in total saccharides:

$$\text{Result} = (P_{OS}/P_{TS}) \times 100$$

Acceptance criteria

For High Fructose Corn Syrup 42%

Total saccharides: NLT 97.0%, expressed as a percentage of total solids. Total saccharides contain monosaccharides and other saccharides as follows.
Monosaccharides: NLT 92.0%
Fructose: 41.0%–50.0%
Other saccharides: NMT 8.0%

For High Fructose Corn Syrup 55%

Total saccharides: NLT 95.0%, expressed as a percentage of total solids. Total saccharides contain monosaccharides and other saccharides as follows.
Monosaccharides: NLT 94.0%
Fructose: 54.0%–60.0%
Other saccharides: NMT 6.0%

IMPURITIES

- **Residue on Ignition** ⟨281⟩**:** NMT 0.05%
- **Limit of Lead**

[Note—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. For digestion, use acid-cleaned, high-density polyethylene, polypropylene, polytef, or quartz tubes. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in borosilicate glass containers. Cleanse glassware before use by soaking in warm 8 N nitric acid for 30 min and rinsing with deionized water. Store final diluted solutions in acid-cleaned plastic or polytef tubes or bottles.]

Matrix modifier solution: 200 mg/mL of magnesium nitrate. Just before use, transfer 1.0 mL of this solution to a 10-mL volumetric flask, and dilute with 5% nitric acid to volume.

Alternate matrix modifier solution: Just before use, add 0.3 mL of commercially available 10,000 µg/mL palladium standard solution and 5 mL of commercially available 10,000 µg/mL magnesium nitrate standard solution to 9.7 mL of 5% nitric acid, and mix well. [Note—*Alternate matrix modifier solution* can be used to replace the *Matrix modifier solution*. Then the air ashing step in the furnace program (see *Table 2*) can be omitted.]

Standard stock solution: Transfer 10.0 mL of lead nitrate stock solution TS to a 100-mL volumetric flask, add 40 mL of water and 5 mL of nitric acid, and dilute with water to volume. Transfer 1.0 mL of this solution to a second 100-mL volumetric flask, dilute with 5% nitric acid to volume, and mix. This solution contains 0.1 µg/mL of lead.

Standard solutions: Transfer portions of *Standard stock solution* to four suitable containers, and dilute with 5% nitric acid to obtain *Standard solutions* having lead concentrations of 100, 50, 25, and 10 ng/mL, respectively.

Sample solution: [Note—It is recommended to perform this procedure in a fume hood. To ensure that a representative subsample of Syrup is used for analysis, ultrasonic and/or vortex mixing of Syrup samples prior to weighing is recommended.] Transfer 1.5 g of Syrup to two digestion tubes, labeled "Sample solution" and "Temperature monitor solution," and add 0.75 mL of nitric acid to each tube. Place a thermometer in the tube labeled "Temperature monitor solution," then use the *Temperature monitor solution* solely to monitor the temperature to be within ranges specified by the method. Warm both solutions slowly to 90°–95° to avoid spattering. Heat until all brown vapors have dissipated and the samples no longer have a rust-colored tint. This typically takes 20–30 min. Allow the samples to cool. Add 0.5 mL of 50% hydrogen peroxide dropwise to both solutions, heat to 90°–95° for 5 min, and cool. Add a second 0.5-mL portion of 50% hydrogen peroxide dropwise to both solutions, and heat to 90°–100° for 5–10 min or until the solutions are clear. Cool, and transfer the *Sample solution* to a 10-mL volumetric flask. Rinse the tube labeled "Sample solution" with 5% nitric acid, add the rinsing to the volumetric flask, dilute with 5% nitric acid to volume, and mix.

Standard blank: 5% Nitric acid

Sample blank: Transfer 1.5 g of water to a digestion tube, and proceed as directed for the *Sample solution*, beginning with "add 0.75 mL of nitric acid".

Instrumental conditions

Mode: Graphite furnace atomic absorption with pyrolytically coated graphite tubes and adequate means of background correction

Lamp: Lead hollow-cathode

Analytical wavelength: Lead emission line of 283.3 nm

Furnace program: See *Table 2*. [Note—The temperature program may be modified to obtain optimum furnace temperatures.]

Table 2

Step	Temperature (°)	Ramp (s)	Hold Time (s)	Gas	Gas Flow Rate (mL/min)	Read (s)
Dry	200	20	30	Argon	300	—
Char (Ash)	750	40	40	Air[a]	300	—
Cool down	20	1	60	Argon	300	—
Atomize	1800	0	10	Argon	Stop flow	10
Clean	2600	1	7	Argon	300	—
Cool down	20	1	5	Argon	300	—

[a] If the *Matrix modifier solution* is used, air ashing must be used in the experiment. If the *Alternate matrix modifier solution* is used, air can be substituted with argon.

If the *Matrix modifier solution* is used, the furnace controller must be able to handle two gas flows to facilitate air ashing. Argon is used as the purge gas for the furnace for all steps but the char. Oxygen ashing is used to avoid build-up of residue during the char step. Breathing quality air is used as the alternative gas for the air ashing. The long (60 s) "Cool down" step prior to atomization ensures that the air used for the oxygen ashing (char) is cleared from the furnace.

Autosampler

Sample volume: 20 µL

Alternative volume: 5 µL of *Matrix modifier solution* (or *Alternate matrix modifier solution*)

Analysis

Samples: Add 5 µL of the *Matrix modifier solution* (or *Alternate matrix modifier solution*) to each 20-µL aliquot of the four *Standard solutions*, add 5 µL of the *Matrix modifier solution* (or *Alternate matrix modifier solution*) to 20 µL of the *Sample solution*, add 5 µL of the *Matrix modifier solution* (or *Alternate matrix modifier solution*) to 20 µL of the *Standard blank*, and add 5 µL of the *Matrix modifier solution* (or *Alternate matrix modifier solution*) to 20 µL of the *Sample blank*.

Use peak area measurements for all quantitations.

Using the *Standard blank* to set the instrument to zero, determine the integrated absorbances of the *Standard solutions*. Plot the integrated absorbances of the *Standard solutions* versus their contents of lead, in ng/mL, and draw the line best fitting the four points to determine the calibration curve. Similarly determine the integrated absorbances of the *Sample solution* and the *Sample blank*. Correct the absorbance value of the *Sample solution* by subtracting from it the absorbance value obtained from the *Sample blank*.

Calculate the concentration of lead, in µg/g, in the portion of Syrup taken:

$$\text{Result} = (V \times C_L/W) \times F$$

V = volume of the *Sample solution*, 10 mL
C_L = concentration of lead in the *Sample solution*, as determined from the calibration curve (ng/mL)
W = weight of Syrup taken to prepare the *Sample solution* (g)
F = conversion factor, 10^{-3} µg/ng

Acceptance criteria: NMT 0.1 µg/g

- **LIMIT OF SULFUR DIOXIDE**

Starch indicator solution: Mix 10 g of soluble starch with 50 mL of cold water. Transfer to 1000 mL of boiling water, stir until completely dissolved, cool, and add 1 g of salicylic acid preservative. [NOTE—Discard the solution after 1 month.]

Sample: 100 g

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.005 N iodine VS

Blank: 200 mL of water

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 250-mL conical flask, add 100 mL of water, and mix. Cool to 5°–10°. While stirring with a magnetic stirrer, add 10 mL of cold (5°–10°) 1.5 N sodium hydroxide. Stir for an additional 20 s, and add 10 mL of *Starch indicator solution*. Add 10 mL of cold (5°–10°) 2.0 N sulfuric acid, and titrate immediately with *Titrant* until a light blue color persists for 1 min. Perform a blank determination, and make any necessary correction.

Calculate the concentration, in µg/g, of sulfur dioxide (SO_2) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F_1]/W\} \times F_2$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F_1 = equivalency factor, 32.0 mg/mEq
W = *Sample* weight (g)
F_2 = conversion factor, 10^3 µg/mg

Acceptance criteria: NMT 30 µg/g

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g.
- **TOTAL SOLIDS**

Analysis: Determine the refractive index of the Syrup at 20° or 45° (see *Refractive Index* ⟨831⟩). Use *Table 3* and *Table 4* for calculating the percentage of total solids on a weight/weight basis. If necessary, interpolate between the refractive index values to find the percentage of total solids to the nearest 0.1%.

Table 3. High Fructose Corn Syrup 42%

Percentage of Total Solids (P_{solid})(%)	Refractive Index at 20°	Refractive Index at 45°
69.0	1.4597	1.4543
70.0	1.4620	1.4565
70.5	1.4632	1.4577
71.0	1.4643	1.4589
72.0	1.4667	1.4612
73.0	1.4691	1.4635
74.0	1.4715	1.4658
75.0	1.4738	1.4683
76.0	1.4763	1.4707
77.0	1.4787	1.4731
78.0	1.4811	1.4755
79.0	1.4836	1.4779
80.0	1.4861	1.4804
81.0	1.4886	1.4829

Table 4. High Fructose Corn Syrup 55%

Percentage of Total Solids (P_{solid})(%)	Refractive Index at 20°	Refractive Index at 45°
75.0	1.4738	1.4680
76.0	1.4762	1.4704
76.5	1.4774	1.4716
77.0	1.4786	1.4728
78.0	1.4811	1.4752
79.0	1.4835	1.4776
80.0	1.4860	1.4801
81.0	1.4885	1.4826

Acceptance criteria

High Fructose Corn Syrup 42%: NLT 70.5%
High Fructose Corn Syrup 55%: NLT 76.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. No storage requirement specified.
- **LABELING:** Label it to state, as part of the official title, the nominal percentage of fructose. Label it to indicate the presence of sulfur dioxide if the residual concentration is greater than 10 µg/g.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Dextrose RS

USP Fructose RS
USP Maltose Monohydrate RS