Food Chemicals Codex | FCC®

Elemental Impurities in Food Ingredients – Pathways to Reducing Levels

# FCC Standards Related to Elemental Impurities – Plans and Opportunities

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# Food Chemicals Codex

## A compendium of internationally recognized standards for the identity and purity of food ingredients

Created by the US-FDA and the US Institute of Medicine in 1966

Currently published by USP, a non-profit organization

A fully independent source of food ingredient standards

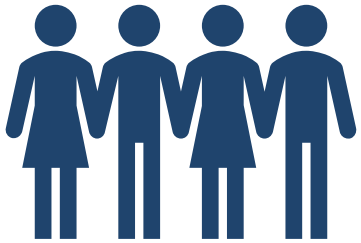
>1250 standards for additives, ingredients, and other food chemicals

Standards are developed by expert volunteers

# USP Foods Expert Volunteers

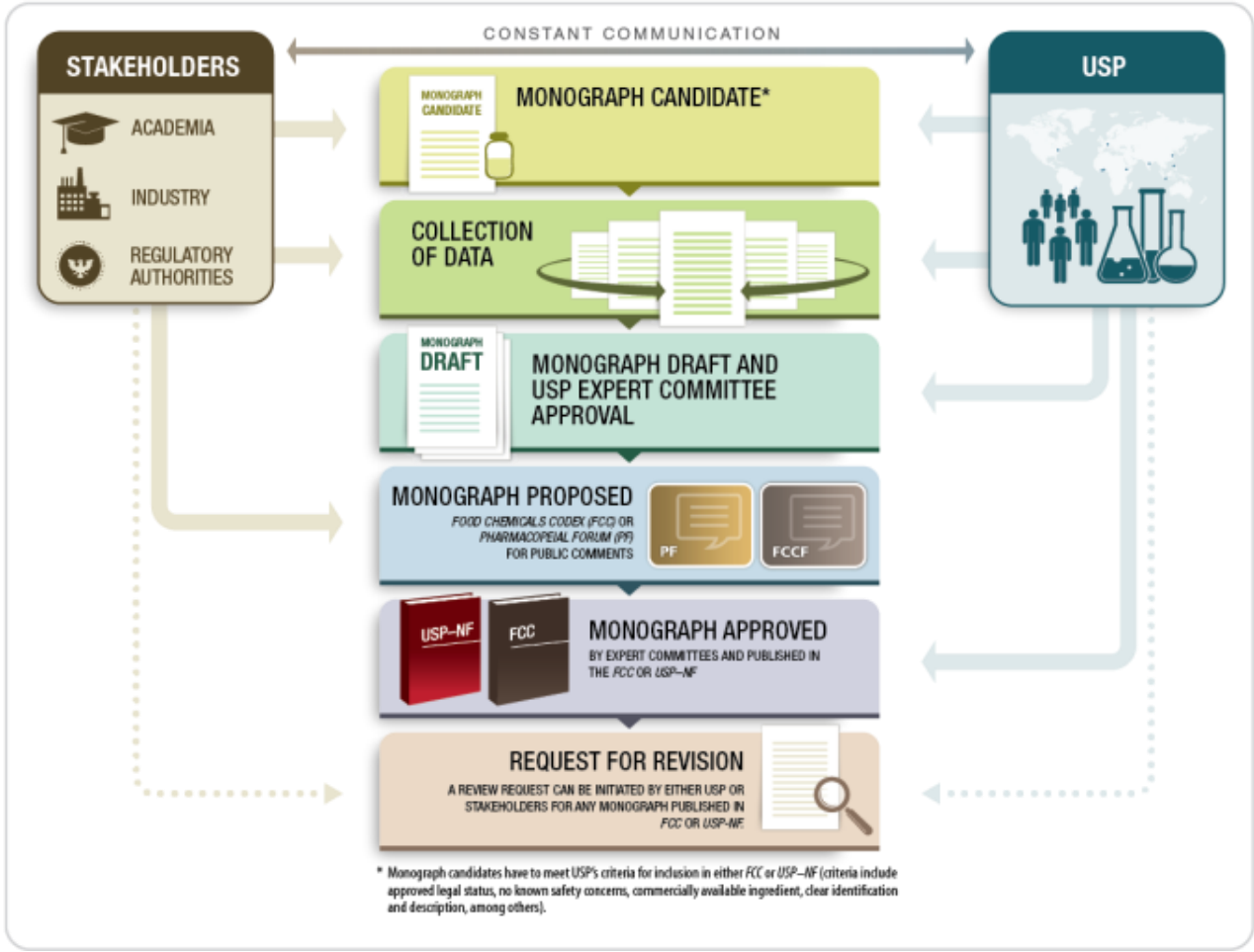
## *USP Food Ingredients Expert Committee*

- USP Olive Oil Authenticity and Quality Expert Panel
- USP High-Value Food Oils Expert Panel
- USP Honey Expert Panel
- USP Dietary Proteins Expert Panel

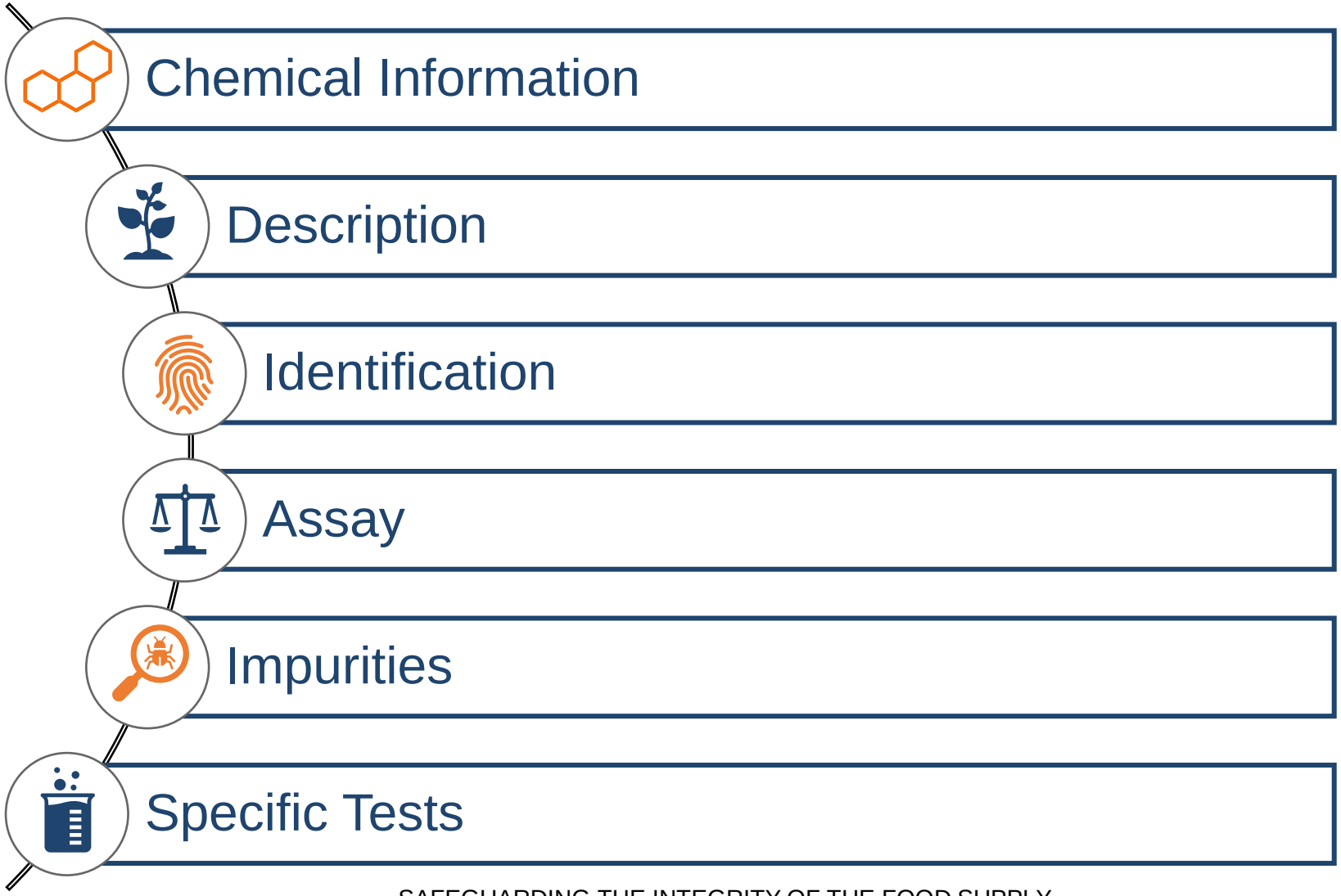


# FCC Standard Setting Process

- An open and transparent process and public participation is encouraged.
- Participation in the revision process results from the support of many individuals and groups
- The FCC Forum publishes twice annually is open to public comment for 90-days
- Public comments received in response to proposed FCC standards are reviewed and considered by the FIEC
- Proposed standards are finalized when the FIEC votes to make them effective text in FCC



# The Elements of an FCC Monograph



# Monograph Example: 2'-Fucosyllactose

***Add the following:***

## ▲2′-FUCOSYLLACTOSE

D-Glucose, *O*-
6-deoxy-α-L-galactopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→4)-

*O*-
6-Deoxy-α-L-galactopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose

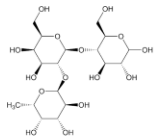
4-*O*-(2-*O*-(6-*O*-Deoxy-α-L-galactopyranosyl)-β-D-galactopyranosyl)-D-glucose

2′-*O*-Fucosyllactose

2′-FL

2′-*O*-L-Fucosyl-D-lactose

Fucosyl-α-1,2-galactosyl-β-1,4-glucose



$C_{18}H_{32}O_{15}$ **Formula wt:** 488.44
CAS RN®: 41263-94-9

### DESCRIPTION

2′-Fucosyllactose occurs as a white to off-white powder or agglomerate. It is produced by fermentation using genetically engineered microorganisms. Following fermentation 2′-Fucosyllactose is purified, concentrated, and dried or crystallized to produce the ingredient of commerce. 2′-Fucosyllactose is a trisaccharide consisting of one molecule each of galactose, glucose, and fucose. It is freely soluble in water.

**Function:** Source of 2′-fucosyllactose; prebiotic

**Packaging and Storage:** Store in sealed bags or containers, protected from light and moisture, in a dry place at room temperature.

### IDENTIFICATION

- **A.** The retention time of the major peak in the chromatogram of *Sample solution 1* corresponds to that of the main peak in the chromatogram of *Standard solution 1*, as obtained in the *Assay*.
- **B. Optical (Specific) Rotation,** *Appendix IIB*
  **Sample solution:** Transfer 5.00 g of sample to a 50-mL volumetric flask, and dissolve in 40 mL of water. Add 0.1 mL of ammonia TS, allow to stand for 30 min, then dilute with water to volume.
  **Acceptance criteria:** $[\alpha]_D^{20}$ between –55.0° and –63.0°

### ASSAY

- **Procedure**
  **Mobile phase:** Acetonitrile, water, and triethylamine (69:31: 0.1, v/v/v)
  **Diluent:** Acetonitrile and water (60:40, v/v)
  **Standard solution 1:** 3.8 mg/mL of USP 2′-Fucosyllactose RS in *Diluent* by using a volumetric flask of at least 10 mL. Sonicate the mixture until the standard is dissolved, then dilute with *Diluent* to volume.
  **Standard solution 2:** 3.4 mg/mL of USP 2′-Fucosyllactose RS in *Diluent* by using a volumetric flask of at least 10 mL. Sonicate the mixture until the standard is dissolved, then dilute with *Diluent* to volume.
  **Standard solution 3:** 0.46 mg/mL of USP 2′-Fucosyllactose RS in *Diluent*. Transfer 3 mL of *Standard solution 1* to a 25-mL volumetric flask and dilute with *Diluent* to volume.
  **System suitability solution 1:** [Note—This procedure gives rise to detectable amounts of 2′-fucosyl-D-lactulose for peak identification.] Weigh 1 mg of D-lactose into a 5-mL volumetric flask. Add approximately 4 mL of *Standard solution 2* as well as 5 µL of triethylamine. Dilute with *Standard solution 2* to volume. Close the flask tightly using a safety clip or similar. Heat the flask for 30 min at 70°. After 30 min, cool the flask down to room temperature.
  **System suitability solution 2:** Transfer 3 mL of *Standard solution 1* to a 100-mL volumetric flask and dilute with *Diluent* to volume.
  **Sample solution 1:** Prepare duplicate solutions containing 4.0 mg/mL of 2′-fucosyllactose in *Diluent* as follows. For each replicate, transfer an amount of sample equivalent to 100 mg of 2′-fucosyllactose on the anhydrous basis to a 25-mL volumetric flask and add about 20 mL of *Diluent* to the flask. Sonicate for about 5 min to dissolve the sample, cool to room temperature, and dilute with *Diluent* to volume.
  [Note—*Sample solution 1* is used in the *Assay*. The dilution provided is based on a sample containing 85%–100% of 2′-fucosyllactose. Adjust the dilution for products with 2′-fucosyllactose outside of this range.]
  **Sample solution 2:** Prepare duplicate solutions containing 40 mg/mL of 2′-fucosyllactose in *Diluent* as follows. For each replicate, transfer an amount of sample equivalent to 1000 mg of 2′-fucosyllactose on the anhydrous basis to a 25-mL volumetric flask and add about 20 mL of *Diluent* to the flask. Sonicate for about 5 min to dissolve the sample, cool to room temperature, and dilute with *Diluent* to volume.
  [Note—*Sample solution 2* is used in the test for *Related Compounds* in *Specific Tests*. The dilution provided is based on a sample containing 0.3%–5% *Related Compounds*. Adjust the dilution for products with *Related Compounds* outside of this range.]
  **Blank:** *Diluent*
  **Chromatographic system,** *Appendix IIA*
  **Mode:** HPLC
  **Detector:** Refractive index
  **Detector cell temperature:** 35°
  **Columns:** Use three 4.6-mm × 250-mm analytical columns with 3.5-µm particles covalently modified with alkyl amide groups (without being endcapped)[1] connected in series. Use a 3.9-mm × 5-mm precolumn with the same particle size and stationary phase.[2]
  **Column temperature:** 60°
  **Flow rate:** 1.2 mL/min
  **Injection volume:** 100 µL
  **Run time:** 35 min
  **System suitability**
  **Samples:** *System suitability solution 1*, *System suitability solution 2*, and *Blank*
  **Suitability requirements**
  **Resolution:** NLT 1.5 between 2′-fucosyl-D-lactulose and D-lactose, *System suitability solution 1*. [Note—Use the Reference Chromatogram provided with USP 2′-Fucosyllactose RS for peak identification.]
  **Relative standard deviation:** NMT 2.0% for the 2′-fucosyllactose peak area for six replicate injections, *Standard solution 1*
  **Signal-to-noise ratio:** NLT 10, *System suitability solution 2*
  **Peak interference:** No peak at the retention time for 2′-fucosyllactose, *Blank*
  **Analysis:** Using this sequence, separately inject *Standard solution 3*, *Standard solution 2*, *Standard solution 1*, *Sample solution 1* (duplicate solutions), and *Sample solution 2* (duplicate solutions) into the chromatograph and record the resulting chromatograms. Use the chromatogram of *Standard solution 1* to identify the peak of 2′-fucosyllactose in the chromatograms of *Sample solution 1*. Generate a standard curve, not forced through the origin, for 2′-fucosyllactose using the peak areas and concentrations of *Standard solution 1* and *Standard solution 2*. For each replicate of *Sample solution 1*, determine the amount in mg/mL of 2′-fucosyllactose in the replicate based on comparison to the standard curve. Calculate the percentage of 2′-fucosyllactose in the portion of the sample taken:

$$\text{Result} = (C_1/C_2) \times 100$$

$C_1$ = concentration of 2′-fucosyllactose in *Sample solution 1*, obtained from the standard curve (mg/mL)

$C_2$ = concentration of *Sample solution 1* on the anhydrous basis (mg/mL)

Determine the average result, in percent, for the two replicates of *Sample solution 1*.
**Acceptance criteria:** NLT 82%, calculated on the anhydrous basis

### IMPURITIES

**Inorganic Impurities**

- **Arsenic,** *Elemental Impurities by ICP, Appendix IIIC*
  **Acceptance criteria:** NMT 0.2 mg/kg, calculated on the anhydrous basis
- **Lead,** *Elemental Impurities by ICP, Appendix IIIC*
  **Acceptance criteria:** NMT 0.1 mg/kg, calculated on the anhydrous basis

### SPECIFIC TESTS

- **Related Compounds**
  **Analysis:** Identify the peaks for L-fucose, D-lactose, 3,2′-difucosyl-D-lactose, 2′-fucosyllactose, and 2′-fucosyl-D-lactulose in the chromatograms of *Sample solution 2* obtained in the *Assay* by comparison to the chromatograms of *Standard solution 1* (for the location of the 2′-fucosyllactose peak) and using the approximate relative retention times listed in *Table 1*.

**Table 1. Approximate Relative Retention Times**

| Compound | Approximate Relative Retention Time |
|---|---|
| L-Fucose | 0.5 |
| 2′-Fucosyl-D-lactulose | 0.8 |
| D-Lactose | 0.9 |
| 2′-Fucosyllactose | 1.0 |
| 3,2′-Difucosyl-D-lactose | 1.3 |

Generate a standard curve of 2′-fucosyllactose using the peak area and concentration of *Standard solution 3* and forcing the calibration curve through the origin. For each replicate of *Sample solution 2*, determine the concentration of L-fucose, D-lactose, 3,2′-difucosyl-D-lactose, and 2′-fucosyl-D-lactulose in the replicate based on comparison to the standard curve, in mg/mL (as 2′-fucosyllactose).
Calculate the percentage of each compound in the portion of the sample taken:

$$\text{Result} = (C_3/C_4) \times 100$$

$C_3$ = concentration of the analyte of interest (as 2′-fucosyllactose) in *Sample solution 2*, obtained from the standard curve (mg/mL)

$C_4$ = concentration of *Sample solution 2* on the anhydrous basis (mg/mL)

Determine the average result for each analyte, in percent and calculated as 2′-fucosyllactose, for the two replicates of *Sample solution 2*.
**Acceptance criteria:** See *Table 2*.

**Table 2**

| Compound(s) | Acceptance Criteria |
|---|---|
| 2′-Fucosyllactose as determined in the *Assay* + L-fucose + D-lactose + 3,2′-difucosyl-D-lactose | NLT 92%, calculated as 2′-fucosyl-lactose on the anhydrous basis |
| D-Lactose | NMT 8.0%, calculated as 2′-fucosyl-lactose on the anhydrous basis |
| 3,2′-Difucosyl-D-lactose | NMT 7.0%, calculated as 2′-fucosyl-lactose on the anhydrous basis |
| L-Fucose | NMT 3.0%, calculated as 2′-fucosyl-lactose on the anhydrous basis |
| 2′-Fucosyl-D-lactulose | NMT 2.0%, calculated as 2′-fucosyl-lactose on the anhydrous basis |

- **Residue on Ignition (Sulfated Ash),** *Appendix IIC*
  **Acceptance criteria:** NMT 2.0%
- **pH,** *pH Determination, Appendix IIB*
  **Sample:** 50 mg/mL
  **Acceptance criteria:** 3.0–7.5
- **Water,** *Water Determination, Karl Fischer Titrimetric Method, Appendix IIB*
  **Acceptance criteria:** NMT 9.0% ▲ *15 (FCC 13)*

[1] Waters XBridge BEH Amide, or equivalent.
[2] Waters XBridge BEH Amide VanGuard cartridge, or equivalent.

# Monograph Example: 2-FL

***Add the following:***

**▲2′-FUCOSYLLACTOSE**

D-Glucose, *O*-6-deoxy-α-L-galactopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→4)-

**Standard solution 3:** 0.46 mg/mL of USP 2′-Fucosyllactose RS in *Diluent*. Transfer 3 mL of *Standard solution 1* to a 25-mL volumetric flask and dilute with *Diluent* to volume.

**System suitability solution 1:** [NOTE—This procedure gives rise to detectable amounts of 2′-fucosyl-D-lactulose for peak identification.] Weigh 1 mg of D-lactose into a 5-mL volumetric flask. Add approximately 4 mL of *Standard*

**Relative standard deviation:** NMT 2.0% for the 2′-fucosyllactose peak area for six replicate injections, *Standard solution 1*

**Signal-to-noise ratio:** NLT 10, *System suitability solution 2*

**Peak interference:** No peak at the retention time for 2′-fucosyllactose, *Blank*

each replicate of *Sample solution 2*, determine the concentration of L-fucose, D-lactose, 3,2′-difucosyl-D-lactose, and 2′-fucosyl-D-lactulose in the replicate based on comparison to the standard curve, in mg/mL (as 2′-fucosyllactose).

Calculate the percentage of each compound in the portion of the sample taken:

## IMPURITIES

## Inorganic Impurities

- **ARSENIC,** *Elemental Impurities by ICP, Appendix IIIC*

  **Acceptance criteria:** NMT 0.2 mg/kg, calculated on the anhydrous basis

- **LEAD,** *Elemental Impurities by ICP, Appendix IIIC*

  **Acceptance criteria:** NMT 0.1 mg/kg, calculated on the anhydrous basis

**PROCEDURE**

**Mobile phase:** Acetonitrile, water, and triethylamine (69:31:0.1, v/v/v)

**Diluent:** Acetonitrile and water (60:40, v/v)

**Standard solution 1:** 3.8 mg/mL of USP 2′-Fucosyllactose RS in *Diluent* by using a volumetric flask of at least 10 mL. Sonicate the mixture until the standard is dissolved, then dilute with *Diluent* to volume.

**Standard solution 2:** 3.4 mg/mL of USP 2′-Fucosyllactose RS in *Diluent* by using a volumetric flask of at least 10 mL. Sonicate the mixture until the standard is dissolved, then dilute with *Diluent* to volume.

**Run time:** 35 min

**System suitability**

**Samples:** *System suitability solution 1*, *System suitability solution 2*, and *Blank*

**Suitability requirements**

**Resolution:** NLT 1.5 between 2′-fucosyl-D-lactulose and D-lactose, *System suitability solution 1*. [NOTE—Use the Reference Chromatogram provided with USP 2′-Fucosyllactose RS for peak identification.]

| | |
|---|---|
| L-Fucose | 0.5 |
| 2′-Fucosyl-D-lactulose | 0.8 |
| D-Lactose | 0.9 |
| 2′-Fucosyllactose | 1.0 |
| 3,2′-Difucosyl-D-lactose | 1.3 |

Generate a standard curve of 2′-fucosyllactose using the peak area and concentration of *Standard solution 3* and forcing the calibration curve through the origin. For

[1] Waters XBridge BEH Amide, or equivalent.

[2] Waters XBridge BEH Amide VanGuard cartridge, or equivalent.

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# FCC Appendix Items Related to Elemental Impurities and Plasma Spectrochemistry

- Appendix III: Chemical Tests and Determinations
  - Lead, Arsenic, Chloride, Sulfate – Limit Tests
  - Flame Atomic Absorption Spectrophotometric Method
  - Atomic Absorption Spectrophotometric Graphite Furnace Method
  - Elemental Impurities by ICP
    - Method I – ICP-OES
    - Method II – ICP-MS

- Appendix II: Physical Tests and Determinations
  - Plasma Spectrochemistry
    - Sample Preparation and Introduction
    - Standard Preparation
    - ICP (AES and MS)

# FCC Appendix Items Related to Elemental Impurities and Plasma Spectrochemistry

- Appendix III: Chemical Tests and Determinations
  - Lead, Arsenic, Chloride, Sulfate – Limit Tests
  - Flame Atomic Absorption Spectrophotometric Method
  - Atomic Absorption Spectrophotometric Graphite Furnace Method
  - Elemental Impurities by ICP
    - Method I – ICP-OES
    - Method II – ICP-MS

**Analytical procedures for Elemental Impurities by ICP recently revised (June 2022 FCCF Proposal)**

- Appendix II: Physical Tests and Determinations
  - Plasma Spectrochemistry
    - Sample Preparation and Introduction
    - Standard Preparation
    - ICP (AES and MS)

# FCC's Plans for Elemental Impurities

- Review of Limits in Monographs
  - Key ingredients (initial focus on ingredients important to vulnerable populations)
  - Create limits based on ingredient use (infant formula vs. general use)
- Review of Appendices
  - Elemental Impurities Procedures (Appendix III: Chemical Tests and Determinations)
  - Plasma Spectrochemistry (Appendix II: Physical Tests and Determinations)
- New Appendix or Appendix content
  - Potentially include guidelines for stakeholders on approaches to reduce elemental impurities
  - New appendix content

# FCC Mechanisms for Collaboration and Request for Feedback

- FCC relies heavily on stakeholder input
- Platforms for providing input
  - Commenting on Proposals
  - Open Forums
  - Stakeholder Forms
  - Workshops

- **Feedback**
  - Where should FCC prioritize its work to have the greatest impact?
  - Technical input and data needed!
  - Other areas where FCC can contribute towards efforts to lower exposure to elemental impurities?

Our mission:
**To grow our partnerships with industry and regulatory stakeholders through collaborative, ongoing dialogue in an open setting with the goal of improving our standards.**

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https://www.usp.org/get-involved/provide-input/stakeholder-forums

# Thank you

# Stay Connected

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