Elemental Impurities in Food Ingredients – Pathways to Reducing Levels

FCC Standards Related to Elemental Impurities – Plans and Opportunities

Eric Schwartz Ph.D.
Senior Scientist I, Food Standards
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

- Chemical Information
- Description
- Identification
- Assay
- Impurities
- Specific Tests
Monograph Example: 2'-Fucosyllactose

**SAFEGUARDING THE INTEGRITY OF THE FOOD SUPPLY**

**Monograph Example: 2'-Fucosyllactose**

**Formula wt: 685.44**

**CAS RN: 41263-96-9**

**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 for use in food. 2'-Fucosyllactose is a disaccharide consisting of one molecule each of galactose and fucose. Its function is to be used as a prebiotic.

**Function**: Sources of 2'-fucosyllactose products

**Piloting and Storage**: Store in sealed bag or container, protected from light and moisture, in a dry place at room temperature.

**IDENTIFICATION**
- The retention time of the major peak in the chromatogram of Sample solution 1 corresponds to that of the main peak (at retention time of Standard solution 1, in duplicate) in the assay.
- **OPTICAL (SPINNING) RESOLUTION**: Appendix A4
  - **Sample solution**: Transfer 5.0 g of sample to a 50-mL volumetric flask, add 90 mL of water, and dilute with water to volume.
  - **Acceptance criteria**: [a]β 5±0.6 and 63.0.

**ASSAY**

**Procedure**
- **Mobile phase**: Acetonitrile, water, and triethylamine (0.31:0.64:0.05, v/v/v)
- **Diluent**: Acetonitrile and water (60:40, v/v)
- **Standard solution 1**: 3.1 mg/mL of USP 2'-Fucosyllactose RS in Diluent by using a volumetric flask of at least 10 mL. Mix the solution until the standard is dissolved, then dilute with Diluent to volume.
- **Standard solution 2**: 0.4 mg/mL of USP 2'-Fucosyllactose RS in Diluent by using a volumetric flask of at least 10 mL. Mix the solution until the standard is dissolved, then dilute with Diluent to volume.
- **Relative standard deviation**: NMT 2.0% for the two replicate samples.
- **Signal to noise ratio**: NLT 10.
- **Peak interference**: NLT 10% peak area for the peak retention time for 2'-Fucosyllactose.

**Analysis**

- Using this method, sequentially inject Standard solution 1, Standard solution 2, and Sample solution 1 (duplicate solution), and Sample solution 2 (duplicate solution) into the chromatographic 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, net forced through the origin, for 2'-fucosyllactose using the peak areas and concentrations 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.

**Result**: $C_i / (C_i + 100)$

- $C_i$ = concentration of 2'-fucosyllactose in Sample solution 1 based on the standard curve (mg/mL)
- $C_{100}$ = concentration of Sample solution 1 on the anthylosis basis (mg/mL)

**IMPURITIES**

- **Arsenic**: Elemental analysis by GC, Appendix A4
  - Acceptance criteria: NMT 0.2 µg/mg, calculated on the anthylosis basis.
- **Lead**: Elemental analysis, Appendix A4
  - Acceptance criteria: NMT 0.1 µg/mg, calculated on the anthylosis basis.

**SPECIFIC TESTS**

- **Relative retention**: Distinguish the peaks for 2'-fucosyllactose and the closely eluting unknown from the chromatograms of Sample solution 1 by comparison to the chromatograms of Standard solution 1. In the chromatogram of Sample solution 1, compare the relative retention times listed in the table below.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Approximate Relative Retention</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.5</td>
<td>NLT 0.5</td>
</tr>
<tr>
<td>Z. P. 4-Sialyllactose</td>
<td>0.8</td>
<td>NMT 1.0</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.9</td>
<td>NMT 1.0</td>
</tr>
<tr>
<td>Z. P. Fucose</td>
<td>1.0</td>
<td>NMT 1.0</td>
</tr>
<tr>
<td>Z. P. 4-O-fucose</td>
<td>1.3</td>
<td>NMT 1.0</td>
</tr>
</tbody>
</table>

**Table 1. Approximate Relative Retention Times**

Generate a standard curve of 2'-fucosyllactose using the peak area and concentration of Standard solution 1, and forcing the calibration curve through the origins. For each replicate of Sample solution 2, determine the concentration of 2'-fucosyllactose, 3'-O-sialyllactose, and 2'-fucosyllactose in the replicate based on comparison to the standard curve, in mg/mL (as 2'-fucosyllactose).

**Acceptance criteria**
NMT 9.0% of 2'-fucosyllactose.
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 A:** 3.16 mg/mL of USP 2'-Fucosyllactose R5 in Diluent, by using a volumetric flask of at least 10 mL. Saturate the mixture until the standard is dissolved, then dilute with Diluent to volume.

**Standard solution B:** 3.4 mg/mL of USP 2'-Fucosyllactose R5 in Diluent, by using a volumetric flask of at least 10 mL. Saturate the mixture until the standard is dissolved, then dilute with Diluent to volume.

**System suitability solution 1:** Prepared by dissolving 100 mg of USP 2'-Fucosyllactose R5 in 1 mL of water. Dilute 1 mL of this solution with water to 25 mL.

**System suitability solution 2:** Prepared by dissolving 100 mg of each of the following in 1 mL of water: USP 2'-Fucosyllactose R5, arabinose, 1,2-O-tetraacetyl-rhamnopyranosyl-(1→2)-rhamnopyranosyl-(1→3)-arabinose, 1,2-O-tetraacetyl-β-D-galactopyranosyl-(1→4)-D-glucose-3,4,6-tri-O-acetyl-2',6'-O-isopropylidene-α-L-rhamnopyranosyl-(1→2)-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→6)-D-glucose. Dilute 1 mL of this solution with water to 25 mL.

**Sample solutions:** Prepare using System suitability solution 2.

**Relative standard deviation:** NMT 2.0% for the peak area in the chromatograms of system suitability solution 1 and standard solution 2.

**Signal-to-noise ratio:** NMT 10 for the peak in the chromatogram of system suitability solution 2 and peak interference in the chromatogram of sample solution 2.

**Peak interference:** No peak should appear at the retention time for USP 2'-Fucosyllactose R5.

**Analysis:** Perform at least four injections of each sample solution using System suitability solution 2.

Generate a standard curve of USP 2'-Fucosyllactose using the peak area and concentration of standard solution 2 and forcing the calibration curve through the origin. For
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)
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Analytical procedures for Elemental Impurities by ICP recently revised (June 2022 FCCF Proposal)
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.

https://www.usp.org/get-involved/provide-input/stakeholder-forums
Thank you
Stay Connected

eric.schwartz@usp.org