Iodixanol Injection

**DEFINITION**
Iodixanol Injection is a sterile solution of Iodixanol in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of Iodixanol (C₃₂H₄₂Cl₂I₆N₆O₁₆), as organically bound iodine. It may contain stabilizers and buffers. It contains no antimicrobial agents.

**IDENTIFICATION**

Delete the following:

- **A. Procedure**
  - Sample: 1 mL of Injection
  - Analysis: Evaporate Sample to dryness, and heat the residue in a crucible.
  - Acceptance criteria: Violet vapors are evolved.

**ASSAY**

- **Procedure**
  - Sample solution: Transfer 2 mL of the Injection to a glass-stoppered, 125-mL conical flask, add 50 mL of 5% sodium hydroxide solution and 1.0 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 1 h. Cool the flask to room temperature, rinse the flask and the filter with several small portions of water, and add the filtered rinsings to the filtrate. Add 20 mL of glacial acetic acid, dilute with water to 200.0 mL, and transfer 100.0 mL of this solution to a 250-mL conical flask.
  - Analysis: Titrate with 0.1 N silver nitrate VS using automatic titration. Each mL of 0.1 N silver nitrate is equivalent to 25.84 mg of CI₃₂H₄₂Cl₂I₆N₆O₁₆. [Note—The result must be corrected for any inorganic halides that may be present due to added stabilizers or buffers.] Acceptance criteria: 95.0%-105.0%

**IMPURITIES**

Delete the following:

- **Inorganic Impurities**
- **Heavy Metals**, Method II (231): NMT 20 ppm

**Change to read:**

- **Organic Impurities, Procedure 1**
  - **Solution A**: Acetonitrile and water (1:1)
  - **Solution B**: Water
  - **Blank solution**: Water
  - **Mobile phase**: See Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>6</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

**Standard stock solution A**: 12.5 mg/mL of anhydrous iodixanol from USP Iodixanol RS in water

**Standard stock solution B**: 0.25 mg/mL of anhydrous iodixanol related compound C from USP Iodixanol Related Compound C RS in water

**Standard stock solution C**: 0.025 mg/mL of anhydrous iodixanol related compound D from USP Iodixanol Related Compound D RS in water

**Standard solution A**: 2.5 mg/mL of Iodixanol obtained from a volume of Injection in water

**Sample solution A**: 25 mg/mL of iodixanol obtained from a volume of Injection in water

**Control solution**: Sample solution A, Standard stock solution B, and water (5:2.5:42.5)

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode**: LC

**Detector**: UV 254 nm

**Column**: 4.6-mm x 25-cm; 5-µm packing L1

**Flow rate**: 1 mL/min

**Injection volume**: 10 µL

**System suitability**

**Samples**: Blank solution, Standard solution A, at least three replicates of Standard solution B, and Control solution

Chromatograph the solutions as mentioned above. The chromatogram from Standard solution A exhibits two or three principal unresolved peaks. If the chromatogram exhibits two principal peaks, their relative areas are 60% and 40%. If the chromatogram exhibits three principal peaks, their relative areas are 60%, 38%, and 2%. The chromatogram from Standard solution B exhibits two resolved peaks due to iodixanol related compound D that elute before the iodixanol peaks and one iodixanol related compound C peak between the two principal iodixanol peaks. The area of the two iodixanol related compound D peaks is between 0.075% and 0.125% of the total area. Add the areas of the two isomer peaks for iodixanol related compound D from each of the three injections of Standard solution B, and calculate the relative standard deviation for the three summed areas: the relative standard deviation is NMT 5%. Measure the height of the iodixanol related compound C peak, and, if necessary, adjust the sensitivity of the amplifier to obtain a peak height between 80% and 100% of the full scale. Measure the height, A, above the baseline of the iodixanol related compound C peak and the height, B, above the baseline of the lowest part of the curve separating this peak from the first principal iodixanol peak: A is NLT 1.38. In the chromatogram from the Control solution, iodixanol related compound C exhibits a measurable peak.

**Analysis**

**Samples**: Blank solution, Sample solution A, and Sample solution B

**High-low chromatography**: Where it is specified to proceed as directed for High-low chromatography, for the chromatogram from Sample solution A, calculate...
Iodixanol

the percentage of each specified related compound in the portion of Injection taken:

{\text{Result} = (10X)/(0.1Y + Z)}

\(X\) = peak area for each of the specified related compounds from Sample solution A

\(Y\) = total area of all the peaks eluted before and after iodixanol from Sample solution A, disregarding any peaks due to injection noise or solvent

\(Z\) = sum of peak areas of iodixanol and any related compounds which are eluted together with, and between, the principal iodixanol peaks from Sample solution B.

Iohexol: If iohexol is present, it exhibits two peaks, with retention times of 0.37 and 0.39 relative to the main iodixanol peak, in the chromatogram from Sample solution A. Draw a baseline at the height of the baseline from the Blank solution.

Calculate the total area of the two peaks and the percentage of iohexol in the portion of Injection taken as directed for High-low chromatography.

\[^{\text{Iohexol related compound A}}_{\text{USP34}}\]: If iohexol related compound A_{USP34} is present, it elutes as a single peak with a retention time of 0.34 relative to the main iodixanol peak, in the chromatogram from Sample solution A. Draw a baseline at the height of the baseline from the Blank solution.

Calculate the area of the peak and the percentage of iohexol related compound A_{USP34} in the portion of Injection taken as directed for High-low chromatography.

Iodixanol related compound C: If iodixanol related compound C is present, only the first and larger peak, with a retention time of 1.07 relative to the main iodixanol peak, is seen between the two principal iodixanol peaks in the chromatogram from Sample solution A; the second iodixanol related compound C peak co-elutes with iodixanol. The area of the first larger peak corresponds to 80% of the total area of iodixanol related compound C. Draw a vertical line through the minimum before the first and larger peak. Draw a horizontal baseline at the minimum after the first and larger peak.

Calculate the percentage of iodixanol related compound C in the portion of Injection taken:

{\text{Result} = 12.5X_j/(0.1Y + Z)}

\(X_j\) = iodixanol related compound C peak area

\(Y\) = total area of all the peaks eluted before and after iodixanol from Sample solution A, disregarding any peaks due to injection noise or solvent

\(Z\) = sum of peak areas of iodixanol and any related compounds which are eluted together with, and between, the principal iodixanol peaks from Sample solution B.

Iodixanol related compound F: If iodixanol related compound F is present, only the first smaller peak with a retention time of 0.8 relative to the main iodixanol peak can be seen in the chromatogram from Sample solution A; the second peak co-elutes with iodixanol. The area of the first and smaller peak corresponds to 25% of the total area of iodixanol related compound F.

Calculate the percentage of iodixanol related compound F in the portion of Injection taken:

{\text{Result} = 40X_j/(0.1Y + Z)}

\(X_j\) = actual observed area of the peak of iodixanol related compound F from Sample solution A

\(Y\) = total area of all the peaks eluted before and after iodixanol from Sample solution A, disregarding any peaks due to injection noise or solvent

\(Z\) = sum of peak areas of iodixanol and any related compounds which are eluted together with, and between, the principal iodixanol peaks from Sample solution B.

Iodixanol related compound G: If iodixanol related compound G is present, the second and larger peak, with a retention time of 0.18 relative to the last iodixanol peak, is seen in the chromatogram from Sample solution A; the first peak co-elutes with iodixanol. The area of the second peak corresponds to 85% of the total area of iodixanol related compound G. Draw the baseline at the height of the baseline from the Blank solution.

Calculate the percentage of iodixanol related compound G in the portion of Injection taken:

{\text{Result} = 10X_j/(0.85(0.1Y + Z))}

\(X_j\) = peak area of iodixanol related compound G

\(Y\) = total area of all the peaks eluted before and after iodixanol from Sample solution A, disregarding any peaks due to injection noise or solvent

\(Z\) = sum of peak areas of iodixanol and any related compounds which are eluted together with, and between, the principal iodixanol peaks from Sample solution B.

Overalkylated related compounds: These compounds elute after iodixanol related compound G, with a retention time greater than 1.18 relative to the last iodixanol peak. Draw the baseline at the height of the baseline from the Blank solution, and determine the peak areas.

Calculate the percentage of overalkylated related compounds as directed for High-low chromatography.

Unspecified related compounds: Examine the chromatograms from Sample solution A and the area of each peak eluting before or after iodixanol, other than those of iodixanol, specified related compounds, specified impurities, and overalkylated related compounds. Draw the baseline at the height of the baseline from the Blank solution.

Calculate the percentage of the largest of these peaks as directed for High-low chromatography.

Other unspecified related compounds: Determine the area of any unspecified peak eluting between those of iodixanol. Draw the baseline between minima, and calculate the percentage as directed for High-low chromatography.

1 \(5\)-(Acetylamino)-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

2 \(2\)-[(3,5-bis[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triodophenyl]amino[methyl]-N,N'-bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4H-1,4-benzoazine-6,8-dicarboxamide.

3 \(4\)-Acetyl-2-[(3,5-bis[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triodophenyl]amino[methyl]-N,N'-bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4H-1,4-benzoazine-6,8-dicarboxamide.
Individual impurities: See Table 2.

<table>
<thead>
<tr>
<th>Name</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodixanol related compound A</td>
<td>0.2</td>
</tr>
<tr>
<td>Iodixanol related compound C.</td>
<td>0.4</td>
</tr>
<tr>
<td>Iodixanol related compound F</td>
<td>0.2</td>
</tr>
<tr>
<td>Iodixanol related compound G</td>
<td>0.2</td>
</tr>
<tr>
<td>Iohexol</td>
<td>0.6</td>
</tr>
<tr>
<td>Overalkylated related compounds</td>
<td>1.0</td>
</tr>
<tr>
<td>Any individual unspecified related compound</td>
<td>0.2</td>
</tr>
<tr>
<td>Total unspecified related compounds</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Total related compounds: From each of the chromatograms from Sample solution A, calculate the percentage of all related compounds as the sum of the results for the peaks appearing between the two principal iodixanol peaks, and the area percent:

Result = \[\frac{(100(\sum Y - \sum X_1 + \sum X_2/0.25 + \sum X_3/0.8 + \sum X_4/0.85))}{10(1.1Y + Z)}\]

\(Y\) = total area of all the peaks eluted before and after iodixanol from Sample solution A, disregarding any peaks due to injection noise or solvent

\(X_1\) = actual observed area of the peak of iodixanol related compound F from Sample solution A

\(X_2\) = peak area of iodixanol related compound G

\(X_3\) = iodixanol related compound C peak area

\(X_4\) = sum of peak areas of iodixanol and any related compounds which are eluted together with, and between, the principal iodixanol peaks from Sample solution B

Total impurities: NMT 1.5%

**Organic Impurities, Procedure 2**

### Solution A: Acetonitrile

### Solution B: Water

### Blank solution: Water

### Mobile phase: See Table 3.

### Table 3

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>25</td>
<td>66</td>
<td>34</td>
</tr>
</tbody>
</table>

**Standard stock solution A:** 12.5 mg/mL of anhydrous iodixanol from USP Iodixanol RS

**Standard stock solution B:** 0.025 mg/mL of anhydrous iodixanol related compound D from USP Iodixanol Related Compound D RS

**Standard stock solution C:** 2.5 mg/mL of anhydrous iodixanol related compound E from USP Iodixanol Related Compound E RS

**Standard solution A:** 2.5 mg/mL of anhydrous iodixanol from Standard stock solution A diluted with water

**Standard solution B:** 2.5 mg/mL of anhydrous iodixanol and 0.0025 mg/mL of anhydrous iodixanol related compound D from Standard stock solution A and Standard stock solution B, respectively, diluted with water

**Standard solution C:** 2.5 mg/mL of anhydrous iodixanol and 0.25 mg/mL of anhydrous iodixanol related compound E from Standard stock solution A and Standard stock solution C, respectively, diluted with water

Sample solution: 2.5 mg/mL of iodixanol from a volume of injection

Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L8

**Flow rate:** 2.5 mL/min

**Injection volume:** 10 µL

System suitability

**Samples:** Standard solution A, three times of Standard solution B, Standard solution C, and Sample solution

The chromatogram from Standard solution A exhibits three principal unresolved peaks: the relative areas are 62%, 35%, and 3%; and the retention time of the last iodixanol peak is NMT 14 min. The chromatogram from Standard solution B exhibits two partially unresolved peaks due to iodixanol related compound D, with relative retention times of 0.33 and 0.39, that elute before the iodixanol peaks: the peak area of iodixanol related compound D is between 0.075% and 0.125% of the total area. Disregard any peak due to the solvent.

Determine the sum of the peak areas of the two isomers of iodixanol related compound D for each of the three chromatograms from Standard solution B.

**Relative standard deviation:** NMT 5%

The chromatogram from Standard solution C exhibits two unresolved peaks due to iodixanol related compound E, with relative retention times of 0.67 and 0.72, that elute before the iodixanol peaks. Adjust the sensitivity of the amplifier so that the peak heights are between 90% and 100% of full scale of the highest peak.

**Resolution, R:** NLT 5.0 between the first and largest iodixanol related compound E peak and the first principal iodixanol peak

**Analysis**

**Samples:** Standard solution A, three times of Standard solution B, Standard solution C, and Sample solution

For the first chromatogram from Standard solution B, adjust the sensitivity of the amplifier to obtain a peak height of 15% of the first and larger peak that corresponds to iodixanol related compound D. Use this sensitivity setting for the subsequent injections.

Compare the retention times of the peaks from Standard solution C to those from the Sample solution. Iodixanol related compound E exhibits two peaks, the second of which may partly overlap with another peak; use only the area of the first and larger peak, which corresponds to 60% of the total area of iodixanol related compound E. Draw a baseline at the height of the baseline from the Blank solution.

Calculate the percentage of iodixanol related compound E by dividing the area from the Sample solution by 0.6 and using area percent.

**Iodixanol related compound H**: Appears as a single peak, with a shoulder, on the tail of the iodixanol peak. Calculate the percentage of iodixanol related compound H by using area percent.

**Acceptance criteria:** NMT 0.3% of iodixanol related compound E is found and NMT 0.6% of iodixanol related compound H is found.

©2011 The United States Pharmacopeial Convention  All Rights Reserved.
SPECIFIC TESTS

• **PH (791):** 6.8–7.7
• **Bacterial Endotoxins Test (85):** NMT 0.2 USP Endotoxin Unit/50 mg of iodine

**Change to read:**

• **Osmolality and Osmolarity, Osmolality (785):** 270–310 mOsmol/kg
• **INJECTIONS (1):** Meets the requirements

**Change to read:**

• **Limit of Free Iodide**
  
  **Sample:** 5.0 mL of Injection
  
  **Analysis:** Transfer Sample to a suitable container, add 2.0 mL of acetic acid solution (1:5 glacial acetic acid in water) and 30 mL of water, and titrate with 0.001 N silver nitrate VS. Each mL of 0.001 N silver nitrate is equivalent to 0.1269 mg of iodine. NMT 15.0 mL of 0.001 N silver nitrate is required.

  **Acceptance criteria:** NMT 0.02% based on the content of iodixanol

ADDITIONAL REQUIREMENTS

**Change to read:**

• **Packaging and Storage:** Preserve in single-dose or multi-dose plastic or Type I glass containers. Store at controlled room temperature, protected from light. Do not freeze.

**Add the following:**

• **Labeling** Label containers of Injection to direct the user to discard any unused portion. The labeling states also...