Status: Currently Official on 15-Feb-2025
Official Date: Official as of 01-Mar-2024
Document Type: USP Monographs
DocId: GUID-8A9AC92A-E32C-4100-8A97-D01EAC5805C5_4_en-US
DOI: https://doi.org/10.31003/USPNF_M41764_04_01
DOI Ref: jzili

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 ($C_{35}H_{44}I_6N_6O_{15}$), as organically bound iodine. It may contain stabilizers and buffers. It contains no antimicrobial agents.

IDENTIFICATION

• **A.** The retention times of the two major peaks of the *Sample solution* correspond to those of *Standard solution B*, as obtained in *Organic Impurities, Procedure 2*.

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, and rinse the condenser with 20 mL of water, adding the rinsing to the refluxed solution. Filter the mixture, rinsing the flask and the filter with several small portions of water, and adding 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 autotitration. Each mL of 0.1 N silver nitrate is equivalent to 25.84 mg of $C_{35}H_{44}I_6N_6O_{15}$.

[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

• **Organic Impurities, Procedure 1**

Solution A: Acetonitrile and water (1:1)

Solution B: Water

Blank solution: Water

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	6	94
30	20	80
70	100	0
80	100	0
81	6	94
90	6	94

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 *Standard stock solution A* in water

Standard solution B: *Standard stock solution A*, *Standard stock solution B*, *Standard stock solution C*, and water (5:2.5:2.5:15)

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

Sample solution B: 2.5 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 × 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 1.3*B*. 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 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 that 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*.

Iohexol related compound A[1]: If iohexol related compound A 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 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 and 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:

$$\text{Result} = 12.5X_2/(0.1Y + Z)$$

X_2 = 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 that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

Iodixanol related compound F[2]: If iodixanol related compound F is present, only the first and 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. Draw the baseline at the height of the baseline from the *Blank solution*.

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

$$\text{Result} = 40X_1/(0.1Y + Z)$$

X_1 = 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 that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

Iodixanol related compound G[3]: If iodixanol related compound G is present, the second and larger peak, with a retention time of 1.18 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:

$$\text{Result} = 10X_3/[0.85(0.1Y + Z)]$$

X_3 = 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 that 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*.

Individual impurities: See *Table 2*.

Table 2

Name	Acceptance Criteria, NMT (%)
Iohexol related compound A	0.2
Iodixanol related compound C	0.4
Iodixanol related compound F	0.2
Iodixanol related compound G	0.2

Name	Acceptance Criteria, NMT (%)
Iohexol	0.6
Overalkylated related compounds	1.0
Any individual unspecified related compound	0.2
Total unspecified related compounds	0.5

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:

$$\text{Result} = [100(Y - X_1 - X_3 + X_1/0.25 + X_2/0.8 + X_3/0.85)]/10(0.1Y + Z)$$

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_3 = peak area of iodixanol related compound G

X_2 = iodixanol related compound C peak area

Z = sum of peak areas of iodixanol and any related compounds that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

Total impurities: NMT 1.5%

Change to read:

• **Organic Impurities, Procedure 2**

Solution A: Acetonitrile

Solution B: Water

Blank solution: Water

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	85	15
25	66	34

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[4]: 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.

SPECIFIC TESTS

- **pH ⟨791⟩:** 6.8–7.5
- **Bacterial Endotoxins Test ⟨85⟩:** NMT 0.2 USP Endotoxin Unit/50 mg of iodine
- **Osmolality and Osmolarity ⟨785⟩**

Osmolality: 270–310 mOsmol/kg

- **Injections and Implanted Drug Products ⟨1⟩:** Meets the requirements
- **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

- **Packaging and Storage:** Preserve in single-dose or multi-dose plastic or preferably Type I glass containers. Store at controlled room temperature, protected from light. Do not freeze.
- **Labeling:** Label containers of Injection to direct the user to discard any unused portion. The labeling states also that it is not to be used if it is discolored or contains a precipitate. Label it to state its routes of administration. Label it to indicate “not approved for intrathecal use”.

Change to read:

- **USP Reference Standards ⟨11⟩**

USP Iodixanol RS

USP Iodixanol Related Compound C RS

5-[Acetyl[3-[[3,5-bis[[(2,3-dihydroxyprop yl)amino]carbonyl]-2,4,6-triiodophenyl]amino]-2-hydroxypropyl]amino]*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

USP Iodixanol Related Compound D RS

5-[Acetyl(2-hydroxy-3-methoxypropyl)amino]-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

USP Iodixanol Related Compound E RS

▲5-{*N*-[3-(*N*-{3-Carbamoyl-5-[(2,3-dihydroxypropyl)carbamoyl]-2,4,6-triiodophenyl}acetamido)-2-hydroxypropyl]acetamido}-N^1,N^3-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide.▲ (ERR 1-Mar-2024)

[1] 5-(Acetylamino)-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

[2] 2-[[Acetyl[3,5-bis[[(2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4*H*-1,4-benzoxazine-6,8-dicarboxamide.

[3] 4-Acetyl-2-[[acetyl[3,5-bis[[(2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4*H*-1,4-benzoxazine-6,8-dicarboxamide.

[4] ▲5-[[3-[[3-[[[3-[[3-[[3,5-Bis-[[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl](acetylamino)]-2-hydroxypropyl](acetylamino)]-5-[[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl]carbonyl]amino]-2-hydroxypropyl]oxy]-2-hydroxypropyl](acetylamino)]-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzendicarboxamide▲ (ERR 1-Mar-2024) ·

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
IODIXANOL INJECTION	Documentary Standards Support	SM42020 Small Molecules 4

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 36(1)

Current DocID: GUID-8A9AC92A-E32C-4100-8A97-D01EAC5805C5_4_en-US
DOI: https://doi.org/10.31003/USPNF_M41764_04_01
DOI ref: jzili