Iohexol

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Expert Committee	Chemical Medicines Monographs 4
Reason for Revision	Compliance

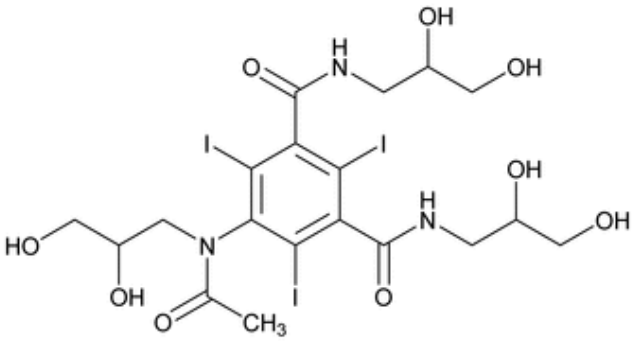
In accordance with the Rules and Procedures of the 2015–2020 Council of Experts, the Chemical Medicines Monographs 4 Expert Committee has revised the Iohexol monograph. The purpose for the revision is to reinstate the statement “Exclude peaks with a relative retention time between 0.84 [relative to the endo-isomer of iohexol (first main peak)] and that of the endo-isomer of iohexol” that was in the official monograph in *USP36–NF31*.

Existing references to reagents have been updated for consistency with the reagent entry names. For additional information about reagent cross references, please see the related Compendial Notice.

The Iohexol Revision Bulletin supersedes the currently official monograph.

Should you have any questions, please contact Ravi Ravichandran, Principal Scientific Liaison (301-279-0434 or rr@usp.org).

Iohexol



$C_{19}H_{26}I_3N_3O_9$ 821.14

1,3-Benzenedicarboxamide, 5-[acetyl(2,3-dihydroxypropyl) amino]-*N,N′*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo; *N,N′*-Bis(2,3-dihydroxypropyl)-5-[*N*-(2,3-dihydroxypropyl) acetamido]-2,4,6-triiodoisophthalamide [66108-95-0].

DEFINITION

Iohexol contains NLT 98.0% and NMT 102.0% of iohexol ($C_{19}H_{26}I_3N_3O_9$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨197K⟩
- **B.** The retention times of the two principal peaks of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Organic Impurities*.

ASSAY

- **Procedure**

Sample: 500 mg of Iohexol

Sample solution: Transfer the *Sample* to a glass-stoppered, 125-mL conical flask. Add 25 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc. Connect the flask to a reflux condenser, and reflux for 1 h. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and pass the mixture through a filter. Rinse the flask and the filter thoroughly with small portions of water, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid.

Titrimetric system
(See *Titrimetry* ⟨541⟩ .)
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with 0.1 N silver nitrate VS.

Calculate the percentage of iohexol ($C_{19}H_{26}I_3N_3O_9$) in the portion of Iohexol taken:

$$\text{Result} = [(V \times N \times F)/W] \times 100$$

V = *Titrant* volume consumed by the *Sample* (mL)
N = *Titrant* normality (mEq/mL)
F = equivalent weight of iohexol, 273.7 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Limit of Ionic Compounds**

Rinse all glassware five times with distilled water.

Standard solution: 0.002 mg/mL of sodium chloride in water
Sample solution: 1 g of Iohexol in 50 mL of water
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The specific conductance of the *Sample solution* is NMT that of the *Standard solution* (equivalent to 0.01% ionic compounds as sodium chloride).

- **Limit of Free Iodide**

Sample: 5 g of Iohexol
Sample solution: Dissolve the *Sample* in 20 mL of water.
Titrimetric system
(See *Titrimetry* ⟨541⟩ .)
Mode: Direct titration
Titrant: 0.001 N silver nitrate VS
Endpoint detection: Potentiometric
Analysis
Calculate the percentage of free iodide in the portion of the *Sample* taken:

$$\text{Result} = [(V \times N \times F)/W] \times 100$$

V = *Titrant* volume consumed by the *Sample* (mL)
N = *Titrant* normality (mEq/mL)
F = equivalent weight of iodide, 126.9 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 0.001%

Change to read:

- **Organic Impurities**

Solution A: Acetonitrile
Solution B: Water
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	1	99
60	13	87

System suitability solution: 1.5 mg/mL of USP Iohexol RS and 0.0075 mg/mL of USP Iohexol Related Compound A RS in water
Sample solution: 1.5 mg/mL of Iohexol
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
Sample: *System suitability solution*

[Note—Iohexol may give two nonresolved peaks due to exo–endo isomerism. In addition, a small peak due to iohexol usually appears at the leading edge of the first principal peak. This small peak has a retention time about 1.2 min less than the first principal peak. The relative retention times for the iohexol related compound A, iohexol endo-isomer, iohexol exo-isomer, and *O*-alkylated compounds peaks are 0.85, 0.96, 1.0, and 1.1–1.4, respectively.]

Suitability requirements
Resolution: NLT 5.0 between iohexol related compound A and the exo-isomer (the second and greater peak) of iohexol
Analysis
Sample: *Sample solution*
Calculate the percentage of *O*-alkylated compounds and any other individual impurity in the portion of Iohexol taken. ▲Exclude peaks with a relative retention time between 0.84 [relative to the endo-isomer of iohexol (first main peak)] and that of the endo-isomer of iohexol.▲ (RB 1-Aug-2019) Disregard any peak less than or equal to 0.03% of the principal peaks.

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity
r_T = sum of all of the peak responses

Acceptance criteria
***O*-alkylated compounds:** NMT 0.6%
Any individual impurity: NMT 0.1%
Total impurities excluding *O*-alkylated compounds: NMT 0.3%

• **LIMIT OF 2-METHOXYETHANOL**
Internal standard solution: 0.01 mg/mL of secondary butyl alcohol in water
Standard stock solution: 0.005 mg/mL of methanol and 0.01 mg/mL each of isopropyl alcohol, secondary butyl alcohol, and 2-methoxyethanol in *Internal standard solution*
Standard solution: Transfer about 0.25 g of USP Iohexol RS and 1.0 mL of *Standard stock solution* to a headspace vial, and seal the vial with a septum and crimp cap.
Sample solution: Transfer about 0.25 g of Iohexol and 1.0 mL of *Internal standard solution* to a headspace vial, and seal the vial with a septum and crimp cap.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: GC with suitable headspace autosampler
Detector: Flame ionization
Column: 0.53-mm × 30-m fused-silica coated with a 1-µm phase G16
Temperatures
Autosampler: 105°
Needle: 130°–140°
Injection port: 150°
Detector: 200°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	3
40	8	100	1

Carrier gas: Helium
Flow rate: 11 mL/min
Injection volume: 1 mL of the headspace
System suitability
Sample: *Standard solution*
[NOTE—The typical relative retention times for methanol, isopropyl alcohol, secondary butyl alcohol, and 2-methoxyethanol are 0.5, 0.6, 1.0, and 1.9, respectively.]
Suitability requirements
Resolution: NLT 1.0 between methanol and isopropyl alcohol
Relative standard deviation: NMT 10.0% for the ratio of 2-methoxyethanol to the internal standard
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of 2-methoxyethanol in the portion of Iohexol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak response ratio of 2-methoxyethanol to the internal standard from the *Sample solution*
R_S = peak response ratio of 2-methoxyethanol to the internal standard from the *Standard solution*
C_S = concentration of 2-methoxyethanol in the *Standard solution* (µg/mL)
C_U = concentration of Iohexol in the *Sample solution* (g/mL)

Acceptance criteria: NMT 20 µg/g of 2-methoxyethanol

• **LIMIT OF 3-CHLOROPROPANE-1,2-DIOL**
Standard solution: 0.025 mg/mL of 3-chloropropane-1,2-diol in ethyl acetate
Sample solution: Transfer 1 g of Iohexol to a separator. Dissolve in 1 mL of water. Extract 4 times with 2 mL of ethyl acetate, and combine the extracts. Dry the combined extracts with anhydrous sodium sulfate. Filter, and wash the filter with a small amount of ethyl acetate. Combine the wash with the filtrate, and concentrate to a volume of 0.7 mL, using a warm water bath and a stream of nitrogen. Dilute with ethyl acetate to 2 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m fused-silica capillary bonded with a 1-µm layer of phase G46
Temperatures
Injection port: 230°
Detector: 250°
Column: See *Table 3*.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	—	80	2
80	15	275	—
275	—	275	2

Carrier gas: Helium at 1 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time of the 3-chloropropane-1,2-diol peak is about 8 min.]
Suitability requirements
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The area of the principal peak from the *Sample solution* is NMT the area of the principal peak from the *Standard solution* (NMT 0.0025%).

• **Limit of Free Aromatic Amine**
Solution A: 3 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in a mixture of propylene glycol and water (70:30)
Standard stock solution: 10 µg/mL of USP Iohexol Related Compound B RS in water
Standard solution: Transfer 5 mL of water and 10.0 mL of the *Standard stock solution* to a 25-mL volumetric flask.
Sample solution: Transfer 200 mg of Iohexol to a 25-mL volumetric flask, add 15 mL of water, and mix to dissolve.
Blank: Add 15 mL of water to a 25-mL volumetric flask.
Instrumental conditions
Mode: Vis
Analytical wavelength: 495 nm
Cell: 5 cm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
In conducting the following steps, keep the flasks in iced water and protected as much as possible from light until all of the reagents have been added.
Treat the *Samples* as follows. Place the flask in an ice bath for 5 min. Add 1.5 mL of 6 N hydrochloric acid, and mix by swirling. Add 1.0 mL of sodium nitrite solution (20 mg/mL), and allow to stand in the ice bath for 4 min. Remove the flask from the ice bath, add 1.0 mL of sulfamic acid solution (40 mg/mL), and swirl gently until gas evolution ceases. [**Caution**—Considerable pressure is produced.] Add 1.0 mL of *Solution A*, dilute with water to volume, and allow to stand for 5 min. Measure the absorbance of the *Standard solution* and *Sample solution* against the *Blank solution*.
Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (NMT 0.05% of free aromatic amine).

SPECIFIC TESTS

• **Color of Solution**
Sample solution: 647.2 mg/mL
Blank: Water
Instrumental conditions
Mode: UV-Vis
Analytical wavelengths: 400, 420, and 450 nm
Cell: 1 cm
Analysis
Samples: *Sample solution* and *Blank*
Pass the *Sample solution* through a filter of 0.22-µm pore size.
Determine the absorbances of the *Sample solution* against the *Blank*.
Acceptance criteria: See *Table 4*.

Table 4

Wavelength (nm)	NMT (au)
400	0.180
420	0.030
450	0.015

• **Water Determination** ⟨921⟩**,** *Method I***:** NMT 4.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, light-resistant containers. Store at room temperature.
• **USP Reference Standards** ⟨11⟩
USP Iohexol RS
USP Iohexol Related Compound A RS
5-(Acetylamino)-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
$C_{16}H_{20}I_3N_3O_7$ 747.06
USP Iohexol Related Compound B RS
5-Amino-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
$C_{14}H_{18}I_3N_3O_6$ 705.02