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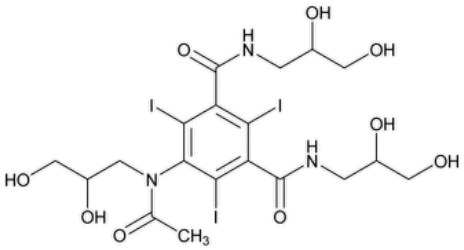
Iohexol



$C_{19}H_{26}I_3N_3O_9$ 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 CAS RN®: 66108-95-0; UNII: 4419T9MX03.

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. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 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 *Titrant*.

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).

Change to read:

• **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▲▲ (USP 1-Dec-2021)

Endpoint detection: Potentiometric

Analysis: Calculate the percentage of free iodide 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 iodide, 126.9 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: NMT 0.001%

• **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. 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%

Change to read:

• **Limit of 2-Methoxyethanol**

Internal standard solution: ▲10 µg/mL▲ (USP 1-Dec-2021) of secondary butyl alcohol in water

▲System suitability solution: 5 µg/mL of methanol and 10 µg/mL each of isopropyl alcohol and 2-methoxyethanol in *Internal standard solution*▲ (USP 1-Dec-2021)

Standard stock solution: ▲10 µg/mL of▲ (USP 1-Dec-2021) 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.

▲Blank solution: Transfer about 0.25 g of USP Iohexol RS and 1.0 mL of *Internal standard solution* to a headspace vial, and seal the vial with a septum and crimp cap.▲ (USP 1-Dec-2021)

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: ▲*System suitability solution*▲ (USP 1-Dec-2021)

[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 ▲secondary butyl alcohol▲ (USP 1-Dec-2021)

Analysis

Samples: *Standard solution*, *Sample solution*, and ▲*Blank solution*▲ (USP 1-Dec-2021)

Calculate the amount of 2-methoxyethanol in the portion of Iohexol taken:

$$\text{Result} = [R_U/(R_S \text{▲} - R_B)]\text{▲ (USP 1-Dec-2021)} \times (C_S/C_U)$$

R_U = peak response ratio of 2-methoxyethanol to ▲secondary butyl alcohol▲ (USP 1-Dec-2021) from the *Sample solution*

R_S = peak response ratio of 2-methoxyethanol to ▲secondary butyl alcohol▲ (USP 1-Dec-2021) from the *Standard solution*

$\text{▲}R_B\text{▲}$ = peak response ratio of 2-methoxyethanol to secondary butyl alcohol from the *Blank solution*▲ (USP 1-Dec-2021)

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

Change to read:

• **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 washings 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 ▲1▲ (USP 1-Dec-2021) 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

Flow rate: 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*.

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

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

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

Chromatographic Database Information: Chromatographic Database

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