Interim Revision Announcement
Official: March 1, 2021

Temozolomide for Injection

DEFINITION

Temozolomide for Injection is a sterile, lyophilized mixture of Temozolomide and suitable added substances. It contains NLT 95.0% and NMT 105.0% of the labeled amount of temozolomide ($C_6H_6N_6O_2$).

[**Caution—**Temozolomide is cytotoxic. Great care should be taken to prevent inhaling particles of temozolomide and exposure to the skin.]

IDENTIFICATION

● **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197A or 197K

Standard: Dissolve a suitable amount of USP Temozolomide RS in methylene chloride, using 2 mL of methylene chloride for each 5 mg of USP Temozolomide RS. Evaporate the methylene chloride under a stream of nitrogen to dryness, and use the residue.

Sample: Inject 40 mL of methylene chloride into the vial of Temozolomide for Injection and shake the vial for 30 min using a mechanical shaker. Allow the vial to sit for 15 min or until undissolved solids settle to the bottom. Pass 15 mL of the supernatant through a filter of 0.45-µm pore size. Discard the first 5 mL and collect the remaining filtrate into a 20-mL scintillation vial. Evaporate the methylene chloride under a stream of nitrogen to dryness, and use the residue.

Analysis

Samples: *Standard* and *Sample*

Acceptance criteria: Meets the requirements

● **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

● **Procedure**

Store the solutions containing temozolomide at 2°–8°.

Solution A: 0.5% (v/v) glacial acetic acid in water

Mobile phase: *Solution A* and methanol (96:4), containing 0.94 g/L of sodium 1-hexanesulfonate (0.005 M)

System suitability stock solution: 0.2 mg/mL of USP Temozolomide RS in *Mobile phase*

System suitability solution: Transfer 50 mL of 0.1 N hydrochloric acid and 50 mL of the *System suitability stock solution* to a container. Heat the container at 80° for 4 h. Transfer 10 mL of this solution into a 25-mL volumetric flask and dilute with *Mobile phase* to volume. [Note—The preparation forms 2-azahypoxanthine, temozolomide acid, and aminoimidazolecarboxamide.]

Standard solution: 0.1 mg/mL of USP Temozolomide RS in *Mobile phase* from the *System suitability stock solution*

Sample stock solution: Nominally equivalent to 2.0 mg/mL of temozolomide in water, prepared as follows. Add 40 mL of water into each of five vials of Temozolomide for Injection and shake manually to dissolve. Combine the solutions from the 5 vials into a 250-mL volumetric flask and dilute with water to volume.

Sample solution: Nominally equivalent to 0.1 mg/mL of temozolomide in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Autosampler temperature: 2°–8°

Flow rate: 1 mL/min

Injection volume: 75 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 1.9 for the temozolomide peak, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of temozolomide ($C_6H_6N_6O_2$) in the portion of Temozolomide for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of temozolomide from the *Sample solution*

r_S = peak response of temozolomide from the *Standard solution*

C_S = concentration of USP Temozolomide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of temozolomide in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

● **Uniformity of Dosage Units ⟨905⟩, *Weight Variation*:** Meets the requirements

IMPURITIES

Change to read:

● **Organic Impurities**

Store the solutions containing temozolomide at 2°–8°.

Mobile phase, System suitability stock solution, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.

Sensitivity solution: 0.1 µg/mL of USP Temozolomide RS in *Mobile phase* from the *System suitability stock solution*

▲**Standard solution:** 1.3 µg/mL of USP Dacarbazine Related Compound A RS in *Mobile phase*

[Note—Dacarbazine related compound A is the hydrochloride salt of aminoimidazolecarboxamide.]▲ (IRA 1-Mar-2021)

Chromatographic system: Proceed as directed in the *Assay*, except for the *Run time*.

Run time: NLT 2.5 times the retention time of the temozolomide peak

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and ▲*Standard solution*▲ (IRA 1-Mar-2021)

Suitability requirements

Resolution: NLT 2.5 between the temozolomide and aminoimidazolecarboxamide peaks, *System suitability solution*

▲**Relative standard deviation:** NMT 5%, *Standard solution*▲ (IRA 1-Mar-2021)

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: ▲*Standard solution* and▲ (IRA 1-Mar-2021) *Sample solution*

▲Calculate the percentage of aminoimidazolecarboxamide in the portion of Temozolomide for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of aminoimidazolecarboxamide from the *Sample solution*

r_S = peak area of dacarbazine related compound A from the *Standard solution*

C_S = concentration of USP Dacarbazine Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of temozolomide in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of aminoimidazolecarboxamide (free base of USP Dacarbazine Related Compound A RS), 126.12

M_{r2} = molecular weight of USP Dacarbazine Related Compound A RS (hydrochloride salt of aminoimidazolecarboxamide), 162.58 ▲ (IRA 1-Mar-2021)

Calculate the percentage of ▲any other▲ (IRA 1-Mar-2021) impurity in the portion of Temozolomide for Injection taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area of ▲any other▲ (IRA 1-Mar-2021) impurity from the *Sample solution*

r_T = sum of the relevant peak areas from the *Sample solution*

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.1%. Disregard any peak due to an excipient.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Azahypoxanthine[a]	0.4	1.6	0.4
Temozolomide related compound A[b,c]	0.5	—	—
Temozolomide acid[d]	0.9	0.76	0.2
Temozolomide	1.0	—	—
Aminoimidazolecarboxamide[e]	1.4	▲—▲ (IRA 1-Mar-2021)	1.0
Cyanotemozolomide[c,f]	2.3	—	—
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	2.0

[a] 4*a*,5-Dihydro-4*H*-imidazo[4,5-*d*][1,2,3]triazin-4-one.

[b] 4-Diazo-4*H*-imidazole-5-carboxamide.

[c] It is a process impurity and is listed for identification only. It is controlled in the drug substance. It is not reported for the drug product and should not be included in the total impurities.

[d] 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carboxylic acid.

[e] 5-Aminoimidazole-4-carboxamide.

[f] 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carbonitrile.

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: Add 40 mL of water into a vial of Temozolomide for Injection and manually shake to dissolve the solids.

Acceptance criteria: 3.0–4.5

- **Bacterial Endotoxins Test** ⟨85⟩**:** Meets the requirements

- **Sterility Tests** ⟨71⟩**:** Meets the requirements
- **Particulate Matter in Injections** ⟨788⟩**:** Meets the requirements for small-volume injections
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩*, Injection Packaging*. Refrigerate at 2°–8°. After reconstitution, store the reconstituted product at room temperature. The reconstituted product must be used within 14 h, including infusion time.

Change to read:

- **USP Reference Standards** ⟨11⟩

▲ USP Dacarbazine Related Compound A RS

5-Aminoimidazole-4-carboxamide hydrochloride.

$C_4H_6N_4O \cdot HCl$ 162.58▲ (IRA 1-Mar-2021)

USP Temozolomide RS

Page Information:

Not Applicable

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