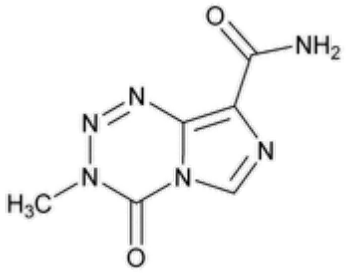
Interim Revision Announcement
Official: March 1, 2021

Temozolomide



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$C_6H_6N_6O_2$ 194.15
Imidazo[5,1-*d*]-1,2,3,5-tetrazine-8-carboxamide, 3,4-dihydro-3-methyl-4-oxo-;
3,4-Dihydro-3-methyl-4-oxoimidazo[5,1-*d*]-*as*-tetrazine-8-carboxamide [85622-93-1]; UNII: YF1K15M17Y.

DEFINITION

Temozolomide contains NLT 98.0% and NMT 102.0% of temozolomide ($C_6H_6N_6O_2$), calculated on the anhydrous basis.

[**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*:** 197K
- **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

[Note—Shake the solutions containing temozolomide to aid the dissolution. Do not sonicate.]

- **Procedure**

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)
Diluent: Dimethyl sulfoxide. [Note—Use a freshly opened bottle.]
Standard solution: 1.0 mg/mL of USP Temozolomide RS in *Diluent*
Sample solution: 1.0 mg/mL of Temozolomide in *Diluent*
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.9
Relative standard deviation: NMT 1.5%
Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of temozolomide ($C_6H_6N_6O_2$) in the portion of Temozolomide taken:

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

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

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

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

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

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

IMPURITIES

● **Residue on Ignition** ⟨281⟩**:** NMT 0.1%

Change to read:

● **Organic Impurities**

[Note—Shake the solutions containing temozolomide to aid the dissolution. Do not sonicate.]

Mobile phase, Diluent, and **Sample solution:** Prepare as directed in the *Assay*.

System suitability solution: Mix 5 mL of 0.1 N hydrochloric acid and 5 mL of 1.0 mg/mL of USP Temozolomide RS in *Diluent*. Heat the container for 1 h on a steam or boiling water bath. [Note—The preparation forms 2-azahypoxanthine, temozolomide acid, and aminoimidazolecarboxamide.]

▲**Standard solution 1:** 1.3 µg/mL of USP Dacarbazine Related Compound A RS in *Diluent*. [Note—Dacarbazine related compound A is the hydrochloride salt of aminoimidazolecarboxamide.]▲ (IRA 1-Mar-2021)

Standard solution ▲2:▲ (IRA 1-Mar-2021) 1.0 µg/mL of USP Temozolomide RS in *Diluent*

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

Run time: NLT 3.2 times the retention time of the temozolomide peak

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between the temozolomide acid and temozolomide peaks, ▲*System suitability solution*

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

Analysis

Samples: *Sample solution*, *System suitability solution*, *Standard solution* ▲*1*, and *Standard solution 2*▲ (IRA 1-Mar-2021)

Inject the *System suitability solution*, and identify the organic impurities according to the relative retention times given in *Table 1*.

▲Calculate the percentage of aminoimidazolecarboxamide in the portion of Temozolomide 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 *Standard solution 1*

C_S = concentration of USP Dacarbazine Related Compound A RS in *Standard solution 1* (mg/mL)

C_U = 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 taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

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

r_S = peak area of temozolomide from *Standard solution* ▲2▲ (IRA 1-Mar-2021)

C_S = concentration of USP Temozolomide RS in *Standard solution* ▲2▲ (IRA 1-Mar-2021) (mg/mL)

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

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

Acceptance criteria: See *Table 1*. [NOTE—Disregard any unspecified impurity peaks less than 0.05%.]

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Azahypoxanthine[a]	0.42	1.6	0.2
Temozolomide related compound A[b]	0.53	1.0	0.5
Temozolomide acid[c]	0.84	1.0	0.1
Temozolomide	1.0	—	—
Aminoimidazolecarboxamide[d]	1.37[e]	▲—▲ (IRA 1-Mar-2021)	0.1
Cyanotemozolomide[f,g] (if present)	2.3	1.0	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.8

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

[d] 5-Aminoimidazole-4-carboxamide. Two peaks may be observed; use the sum of the peak areas for calculation.

[e] It may vary and depend on the column.

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

[g] If possible from the manufacturing process.

SPECIFIC TESTS

● **Water Determination** ⟨921⟩**,** *Method I, Method Ic*: NMT 0.4%

ADDITIONAL REQUIREMENTS

● **Packaging and Storage:** Preserve in well-closed containers, and store at room temperature.

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

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