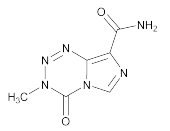
Temozolomide



$C_6H_6N_6O_2$ 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].

DEFINITION

Temozolomide contains NLT 98.0% and NMT 102.0% of temozolomide ($C_6H_6N_6O_2$), calculated on the as-is basis.
[**CAUTION—**Temozolomide is cytotoxic. Great care should be taken to prevent inhaling particles of Temozolomide and exposure to the skin.]

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**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 from the *Sample solution*
r_S = peak area 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 as-is basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 30 ppm

Change to read:

- **ORGANIC IMPURITIES**

[NOTE—Shake the solutions containing temozolomide to aid the dissolution. Do not sonicate.]
Mobile phase, Diluent, and **Sample solution:** Proceed as directed in the *Assay*.
Standard solution: 2.0 µg/mL each of USP Temozolomide RS and USP Dacarbazine Related Compound A RS in *Diluent*
System suitability solution: 0.5 µg/mL each of USP Temozolomide RS and USP Dacarbazine Related Compound A RS in *Diluent* from the *Standard solution*
Peak identification 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 dacarbazine related compound A.]
Chromatographic system: Proceed as directed in the *Assay*, using a run time of NLT 3.2 times the retention time of the temozolomide peak.
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between the temozolomide and dacarbazine related compound A peaks, *Standard solution*
Relative standard deviation: NMT 10% for both the dacarbazine related compound A and temozolomide peaks, *System suitability solution*
Analysis
Samples: *Sample solution*, *Standard solution*, and *Peak identification solution*
Inject the *Peak identification solution*, and identify the organic impurities according to the relative retention times given in *Table 1*.
Calculate the percentage of dacarbazine related compound A (free base) 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 dacarbazine related compound A 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 = concentration of Temozolomide in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dacarbazine related compound A (free base), 126.12
M_{r2} = molecular weight of dacarbazine related compound A (hydrochloride salt), 162.58

Calculate the percentage of any other individual 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 each impurity 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)
F = relative response factor for each individual impurity (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	—	—
Dacarbazine related compound A (free base)[d]	1.37	—	0.1
●Cyanotemozolomide[e,f] (if present)	2.3	1.0	0.15● (RB 1-Jun-2013)

[a] 4a,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. It is a free base of dacarbazine related compound A.
●[e] 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carbonitrile.
[f] If possible from the manufacturing process.● (RB 1-Jun-2013)

Table 1 *(Continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.8

[a] 4a,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. It is a free base of dacarbazine related compound A.
●[e] 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carbonitrile.
[f] If possible from the manufacturing process.● (RB 1-Jun-2013)

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method Ic* ⟨**921**⟩**:** NMT 0.4%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Dacarbazine Related Compound A RS
  5-Aminoimidazole-4-carboxamide hydrochloride.
  $C_4H_6N_4O \cdot HCl$ 162.58
  USP Temozolomide RS