**Temozolomide**

![Chemical Structure of Temozolomide]

C₆H₆N₆O₂  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 (C₆H₆N₆O₂), 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:

\[
\text{Result} = \left(\frac{r_U}{r_S}\right) \times \left(\frac{C_S}{C_U}\right) \times 100
\]

\(r_U\) = 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 aminoimidazolcarboxamide.]

▲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: 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:

\[
\text{Result} = \left(\frac{r_U}{r_S}\right) \times \left(\frac{C_S}{C_U}\right) \times \left(\frac{M_{r1}}{M_{r2}}\right) \times 100
\]

\(r_U\) = 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)
\[ C_U = \text{concentration of Temozolomide in the Sample solution (mg/mL)} \]

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

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

Calculate the percentage of any other impurity in the portion of Temozolomide taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times \left( \frac{1}{F} \right) \times 100
\]

\[ r_U = \text{peak area of any other impurity from the Sample solution} \]

\[ r_S = \text{peak area of temozolomide from Standard solution} \]

\[ C_S = \text{concentration of USP Temozolomide RS in Standard solution (mg/mL)} \]

\[ C_U = \text{concentration of Temozolomide in the Sample solution (mg/mL)} \]

\[ F = \text{relative response factor (see Table 1)} \]

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Azahypoxanthine(^a)</td>
<td>0.42</td>
<td>1.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Temozolomide related compound (^b)</td>
<td>0.53</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Temozolomide acid (^c)</td>
<td>0.84</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Temozolomide</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Aminoimidazolecarboxamide(^d)</td>
<td>1.37(^e)</td>
<td>—</td>
<td>0.1</td>
</tr>
<tr>
<td>Cyanotemozolomide (^f,g) (if present)</td>
<td>2.3</td>
<td>1.0</td>
<td>0.15</td>
</tr>
<tr>
<td>Any unspecified impurity</td>
<td>—</td>
<td>1.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Total impurities</td>
<td>—</td>
<td>—</td>
<td>0.8</td>
</tr>
</tbody>
</table>

\(^a\) 4a,5-Dihydro-4H-imidazo[4,5-\(d\)][1,2,3]triazin-4-one.

\(^b\) 4-Diazo-4H-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**