

BRIEFING

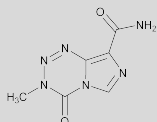
**Temozolomide.** A new USP Pending Monograph is being proposed based on validated methods of analysis. The liquid chromatographic procedures used in the *Assay* and in the test for *Organic Impurities* are based on analyses performed with an Inertsil ODS, 3V, 5- $\mu$ m brand of L1 column. The typical retention time for temozolomide is about 10 min under the conditions for the *Assay* and about 11 min under the conditions for the test for *Organic Impurities*.

(MD-ODD: F. Mao.) RTS—C76065

**Add the following:**

**► Temozolomide**

Draft 1



C<sub>6</sub>H<sub>6</sub>N<sub>6</sub>O<sub>2</sub> 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 C<sub>6</sub>H<sub>6</sub>N<sub>6</sub>O<sub>2</sub>, 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. 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**

• **PROCEDURE**

[NOTE—Use freshly prepared solutions.]

**Solution A:** 0.5% of glacial acetic acid in water

**Mobile phase:** *Solution A* and methanol (9:1)

**Standard solution:** 0.25 mg/mL of USP Temozolomide RS in *Mobile phase*

**Sample solution:** 0.25 mg/mL of Temozolomide in *Mobile phase*

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 254 nm

**Autosampler temperature:** 15°

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.0 mL/min

**Injection size:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Tailing factor:** Between 0.8 and 1.5

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>6</sub>H<sub>6</sub>N<sub>6</sub>O<sub>2</sub> in the portion of Temozolomide taken:

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

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response 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**

**Inorganic Impurities**

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **HEAVY METALS** *Method II* (231): NMT 10 ppm

**Organic Impurities**

• **PROCEDURE**

[NOTE—Use freshly prepared solutions.]

**Solution A:** Proceed as directed in the *Assay*.

**Solution B:** Methanol

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
8.0	90	10
25.0	50	50
40.0	20	80
50.0	10	90
55.0	90	10
60.0	90	10

**Diluent:** *Solution A* and *Solution B* (4:1)

**Standard solution:** 1.0  $\mu$ g/mL of USP Temozolomide RS in *Diluent*

**System suitability solution:** Transfer known amounts of USP Temozolomide Related Compound B RS and USP Temozolomide Related Compound C RS into a suitable volumetric flask. Add methanol equivalent to 10% of the final volume, mix and dissolve. Transfer known amounts of USP Temozolomide RS, USP Dacarbazine Related Compound A RS and USP Temozolomide Related Compound A RS into the same flask, mix and dissolve. Dilute with *Diluent* to volume to obtain a solution containing 0.1 mg/mL each of USP Temozolomide RS, USP Dacarbazine Related Compound A RS, USP Temozolomide Related Compound A RS, USP Temozolomide Related Compound B RS, and USP Temozolomide Related Compound C RS.

**Sample solution:** 1.0 mg/mL of Temozolomide in *Diluent*

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 240 nm and UV 254 nm

[NOTE—Use 240 nm for detection of temozolomide related compound C. Use 254 nm for detection of dacarbazine related compound A, temozolomide related compound A, temozolomide related compound B, and any unspecified impurity.]

2 / Temozolomide

**Autosampler temperature:** 15°

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1.0 mL/min

**Injection size:** 20 μL

**System suitability** [NOTE—Use 254 nm.]

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

**Suitability requirements**

**Resolution:** NLT 8.0 between temozolomide related compound A and temozolomide, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each 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 response of each impurity from the *Sample solution*

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

$C_S$  = concentration of temozolomide 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 *Impurity Table 1*

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*. [NOTE—Disregard any unspecified impurity peaks less than 0.05%.]

**Total impurities:** NMT 0.50%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dacarbazine related compound A	0.21	1.8	0.15
Temozolomide related compound A	0.63	0.81	0.15

**Impurity Table 1** (continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Temozolomide	1.00	—	—
Temozolomide related compound B	3.45	1.4	0.15
Temozolomide related compound C	4.15	1.2	0.15
Any unspecified impurity	—	1.0	0.10

**SPECIFIC TESTS**

- **WATER DETERMINATION, Method I (921):** NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers at a temperature not exceeding 25°.

• **USP REFERENCE STANDARDS (11)**

USP Dacarbazine Related Compound A RS  
[5-Aminoimidazole-4-carboxamide hydrochloride]  
(C<sub>4</sub>H<sub>6</sub>N<sub>4</sub>O · HCl 162.58)

USP Temozolomide RS

USP Temozolomide Related Compound A RS  
[4-Diazo-4H-imidazole-5-carboxamide]  
(C<sub>4</sub>H<sub>3</sub>N<sub>5</sub>O 137.10)

USP Temozolomide Related Compound B RS  
[3-Methyl-1,1-diphenylurea]  
(C<sub>14</sub>H<sub>14</sub>N<sub>2</sub>O 226.27)

USP Temozolomide Related Compound C RS  
[Diphenylcarbamic chloride]  
(C<sub>13</sub>H<sub>10</sub>ClNO 231.68)

◀ (1-May-2010)