Interim Revision Announcement
Official: March 1, 2021

Temozolomide Capsules

Change to read:

DEFINITION
Temozolomide Capsules contain NLT 90.0% and NMT 110.0% 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.]▲ (IRA 1-Mar-2021)

IDENTIFICATION

• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩

Standard solution: 1 mg/mL of USP Temozolomide RS in acetonitrile

Sample solution: Transfer the contents of Capsules indicated in *Table 1* to a glass beaker containing a stir bar. Add acetonitrile according to *Table 1*. Stir for NLT 20 min. Sonicate for 10 min followed by a 30-s stirring to disperse any remaining solids. Allow the contents of the flask to settle.

Table 1

Capsule Strength (mg/Capsule)	Acetonitrile (mL)	Number of Capsules
5	50	4
20	50	1
100	100	1
140	140	1
180	180	1
250	250	1

Chromatographic system
(See *Chromatography ⟨621⟩, General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 20-cm × 20-cm or 10-cm × 20-cm of 0.25-mm layer of chromatographic silica gel mixture

Application volumes

Standard solution: 10 µL

Sample solution: See *Table 2*.

Table 2

Capsule Strength (mg/Capsule)	Sample Solution (µL)
5	25
20	25
100	10
140	10
180	10
250	10

Developing solvent system: Acetone and toluene (90:15)

Analysis: Spot the *Standard solution* and *Sample solution* approximately 2.5 cm from the bottom of the plate. Evaporate the solvent using a current of air. Place the plate in the developing chamber and allow the *Developing solvent system* to rise to approximately 75% of the plate height. Remove the plate from the developing chamber, mark the position of the solvent front, and allow the plate to dry in the fume hood. Observe the plate under 254 nm of UV light.

Acceptance criteria: The R_F value from the *Sample solution* corresponds to that from the *Standard solution*.

• **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 4°.

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: 0.4 mg/mL of USP Temozolomide RS in *Mobile phase*

System suitability solution: Transfer 25 mL of 0.1 N hydrochloric acid and 25 mL of *System suitability stock solution* into a container. Heat the container at 80° for 4 h. [Note—The preparation forms 2-azahypoxanthine, temozolomide acid, and aminoimidazolecarboxamide.]

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

Sample stock solution: Place 10 Capsules in a suitable volumetric flask according to *Table 3*. Add 80% of the final volume of *Mobile phase* with agitation or stirring for NLT 1 h. Ensure that the Capsules are broken apart into small pieces and that contents are adequately dissolved. Dilute with *Mobile phase* to volume and mix well. Allow to sit for 30 min and mix again. Centrifuge 25 mL of this solution for 10 min or until the solution is clear. Pass the supernatant through a suitable filter of 0.45-µm pore size and discard the first few milliliters.

Table 3

Capsule Strength (mg/Capsule)	Volumetric Flask Size (mL)	Nominal Concentration (mg/mL)
5	500	0.1
20	200	1.0
100	1000	1.0
140	2000	0.7
180	2000	0.9
250	2000	1.25

Sample solution: Nominally equivalent to 0.1 mg/mL of temozolomide in *Mobile phase* from *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: 4°

Flow rate: 1 mL/min

Injection volume: 20 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 Capsules 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: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution ⟨711⟩**

Medium: Water; 500 mL (for 5 mg/Capsule), 900 mL (for all other strengths)

Apparatus 1: 100 rpm

Time: 30 min

▲Determine the percentage of the labeled amount of temozolomide ($C_6H_6N_6O_2$) dissolved by using one of the following procedures.

Spectrophotometric procedure▲ (IRA 1-Mar-2021)

Standard solution: Prepare a solution of USP Temozolomide RS in *Medium* according to *Table 4*.

Table 4

Capsule Strength (mg/Capsule)	Nominal Concentration (mg/mL)
5	0.01
20	0.022
100	0.11
140	0.16
180	0.20
250	0.28

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.8-µm pore size. Discard the first few milliliters of the filtrate and use the remaining filtrate for analysis.

Instrumental conditions

▲(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)▲ (IRA 1-Mar-2021)

Mode: UV

Analytical wavelength: Maximum absorbance at about 328 nm

Cell length: See *Table 5*.

Table 5

Capsule Strength (mg/Capsule)	Cell Length (cm)
5	1
20	1
100	0.1
140	0.05
180	0.05
250	0.05

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of temozolomide ($C_6H_6N_6O_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

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

V = volume of *Medium*; 500 mL (for 5 mg/Capsule), 900 mL (for all other strengths)

L = label claim (mg/Capsule)

▲**Chromatographic procedure**

Solution A: 0.4 g/L of ammonium formate in water

Mobile phase: Methanol and *Solution A* (1:99)

Diluent: 0.1% (v/v) glacial acetic acid in water

Standard stock solution: 0.25 mg/mL of USP Temozolomide RS in *Medium*. Sonicate to dissolve.

Standard solution: 0.005 mg/mL of USP Temozolomide RS prepared as follows. Transfer 2 mL of *Standard stock solution* to a 100-mL volumetric flask and dilute with *Diluent* to volume.

Sample stock solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard a few milliliters of the filtrate and use the remaining filtrate.

Sample solution: Dilute *Sample stock solution* with *Diluent* to a concentration that is similar to the *Standard solution*.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

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

Autosampler temperature: 5°

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of temozolomide ($C_6H_6N_6O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (1/L) \times 100$$

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)

V = volume of *Medium*; 500 mL (for 5 mg/Capsule), 900 mL (for all other strengths)

D = dilution factor of the *Sample solution*

L = label claim (mg/Capsule) ▲ (IRA 1-Mar-2021)

Tolerances: NLT 80% (Q) of the labeled amount of temozolomide ($C_6H_6N_6O_2$) is dissolved.

• **Uniformity of Dosage Units ⟨905⟩:** Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**

Store the solutions containing temozolomide at 4°.

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 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 Capsules 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 Capsules taken:

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

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*

Acceptance criteria: See *Table 6*. The reporting threshold is 0.1%. Disregard any peak due to an excipient.

Table 6

Name	Relative Retention Time	Acceptance Criteria, NMT (%)	
		5 mg/Capsule or 20 mg/Capsule	All Other Strengths
2-Azahypoxanthine[a]	0.4	0.8	0.8
Temozolomide related compound A[b,c]	0.5	—	—
Temozolomide acid[d]	0.9	0.2	0.2
Temozolomide	1.0	—	—
Aminoimidazolecarboxamide[e]	1.4	1.2	1.0
Cyanotemozolomide[c,f]	2.3	—	—
Any unspecified impurity	—	0.2	0.2
Total impurities	—	1.7	1.2

[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

● **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count is NMT 10^3 cfu/g, and the total combined molds and yeasts count is NMT 5×10^2 cfu/g. It meets the requirements of the tests for the absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

● **Packaging and Storage:** Preserve in tight containers, and store at controlled 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

Page Information:

Not Applicable

DocID:

© 2020 The United States Pharmacopeial Convention *All Rights Reserved*.