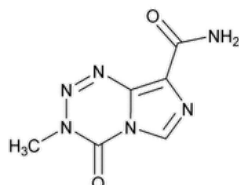


Status: Currently Official on 16-Feb-2025
 Official Date: Official as of 01-Mar-2021
 Document Type: USP Monographs
 DocId: GUID-AB82345A-67C8-4E20-924A-2396C13CFB5C_5_en-US
 DOI: https://doi.org/10.31003/USPNF_M1434_05_01
 DOI Ref: 6hr7m

© 2025 USPC
 Do not distribute

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 CAS RN®: 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)



Documents

Document Sharing Hub