Edition: BP 2025 (Ph. Eur. 11.6 update)

# Temozolomide for Injection

General Notices

**Action and use**

Antineoplastic alkylating agent.

## DEFINITION

Temozolomide for Injection is a sterile material consisting of Temozolomide with or without excipients. It is supplied in a sealed container.

*The contents of the sealed container comply with the requirements for Powder for Injections or Infusions stated under Parenteral Preparations and with the following requirements.*

**Content of temozolomide, $C_6H_6N_6O_2$**

95.0 to 105.0% of the stated amount.

## IDENTIFICATION

To one vial, add 40 mL of *dichloromethane* and shake for 30 minutes. Invert and allow to stand until undissolved solids settle. Filter 15 mL of the clear solution (a 0.45-µm PTFE filter is suitable). Discard the first 5 mL and evaporate the remaining filtrate to dryness under a stream of *nitrogen*. The infrared absorption spectrum of the residue, Appendix II A, is concordant with the reference spectrum of temozolomide *(RS 500)*.

## TESTS

**Related substances**

Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

(1) Dissolve the contents of a sealed container in sufficient water to produce a solution containing 0.2% w/v of Temozolomide. Dilute 1 volume of this solution to 20 volumes with mobile phase and mix.
(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.
(3) 0.1% w/v of *temozolomide for peak identification EPCRS* in the mobile phase.
(4) Dissolve 10 mg of *temozolomide BPCRS* in 25 mL of mobile phase and 25 mL of 0.1M *hydrochloric acid*. Mix and heat the solution at 80° for 4 hours (generation of impurities A, B and E).
(5) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS-2 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.
(e) Use a detection wavelength of 270 nm.
(f) Inject 75 µL of each solution.
(g) Allow the chromatography to proceed for twice the retention time of temozolomide.

MOBILE PHASE

A 0.094% w/v solution of *sodium hexanesulfonate* in a mixture of 4 volumes of *methanol* and 96 volumes of a 0.5% v/v of *glacial acetic acid*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to temozolomide (retention time, about 9 minutes) are: impurity E, about 0.4; impurity D, about 0.5, impurity B, about 0.9 and impurity A, about 1.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the resolution between the peaks due to temozolomide and impurity A is not less than 2.5.

LIMITS

In the chromatogram obtained with solution (1), identify any peak due to impurity D using the chromatogram obtained using solution (3) and any peaks due to impurities A and E using the chromatogram obtained using solution (4). Multiply the areas of any peaks due to impurities A and E by the corresponding correction factors: impurity A, 0.4 and impurity E, 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity D is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity E is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (5) (0.2%);

the sum of the areas of all *secondary peaks*, excluding impurities A and D, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

## ASSAY

Carry out the method for liquid chromatography, Appendix III D, using the following solutions. Store the solutions at 4°.

(1) Dissolve the contents of a sealed container in sufficient *water* to produce a solution containing 0.2% w/v of Temozolomide. Dilute 1 volume of this solution to 20 volumes with mobile phase and mix.
(2) 0.01% w/v of *temozolomide BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described in the test for Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (2), the symmetry factor is not greater than 1.9.

DETERMINATION OF CONTENT

Calculate the content of temozolomide, $C_6H_6N_6O_2$, in the sealed container from the chromatograms obtained and using the declared content of $C_6H_6N_6O_2$, in *temozolomide BPCRS*. Repeat the procedure with a further nine sealed containers. Calculate the average content of $C_6H_6N_6O_2$ per container from the 10 individual results thus obtained.

## IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Temozolomide.