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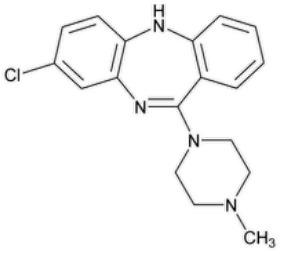
Clozapine



$C_{18}H_{19}ClN_4$ 326.83

5*H*-Dibenzo[*b*,*e*][1,4]diazepine, 8-chloro-11-(4-methyl-1-piperazinyl)-;

8-Chloro-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine CAS RN®: 5786-21-0.

DEFINITION

Clozapine contains NLT 98.0% and NMT 102.0% of clozapine ($C_{18}H_{19}ClN_4$), calculated on the dried basis.

IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K or 197A
- **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**

Mobile phase: Methanol and water (80:20). To each liter add 0.75 mL of triethylamine.

Diluent: Methanol and water (80:20)

System suitability stock solution: Transfer 10 mg of USP Clozapine RS to a suitable container, add 5 mL of 0.1 N hydrochloric acid VS and heat for 2 h at 90°. Transfer this solution to a 100-mL volumetric flask, add 15 mL of water, and dilute with methanol to volume.

Standard solution: 0.1 mg/mL of USP Clozapine RS in *Diluent*

System suitability solution: *System suitability stock solution* and *Standard solution* (50:50)

Sample solution: 0.1 mg/mL of Clozapine in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 257 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 3 times the retention time of clozapine

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between clozapine and any other peak, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of clozapine ($C_{18}H_{19}ClN_4$) in the portion of Clozapine taken:

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

r_U = peak response of clozapine from the *Sample solution*

r_S = peak response of clozapine from the *Standard solution*

C_S = concentration of USP Clozapine RS in the *Standard solution* (mg/mL)

C_U = concentration of Clozapine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.1%

Change to read:

• **Organic Impurities**

Buffer: 2.0 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.4. [Note—The pH of this solution must not be below 2.4.]

Solution A: Acetonitrile, methanol, and *Buffer* (10:10:80)

Solution B: Acetonitrile, methanol, and *Buffer* (40:40:20)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
24	0	100
29	0	100
40	100	0

Diluent: Methanol and water (80:20)

System suitability solution: Dissolve 4 mg of USP Clozapine Resolution Mixture RS in 4 mL of methanol, add 1 mL of water, and dilute with *Diluent* to 10 mL.

Standard solution: 0.75 µg/mL of USP Clozapine RS in *Diluent*

Sample solution: 750 µg/mL of Clozapine prepared as follows. Transfer a suitable quantity of Clozapine to a suitable volumetric flask. Dissolve in 80% of the flask volume of methanol, sonicate for about 3 min, and dilute with water to volume.

Chromatographic system

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

Mode: LC

Detector: UV 257 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

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

[Note— See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.5 between demethyl clozapine and clozapine, *System suitability solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each related compound and any unknown impurity in the portion of Clozapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any impurity from the *Sample solution*

r_S = peak response of clozapine from the *Standard solution*

C_S = concentration of USP Clozapine RS from the *Standard solution* (µg/mL)

C_U = concentration of Clozapine from the *Sample solution* (µg/mL)

F = relative response factor of the impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Demethyl ▲clozapine▲ (ERR 1-Jul-2021) [a]	0.9	1.0	0.3
Clozapine	1.0	—	—
Benzoyl methylpiperazine analog [b]	1.1	0.35	0.2
Chlorodibenzodiazepinone [c]	1.6	1.2	0.1
Didiazepinyl piperazine [d]	1.7	1.0	0.2
Individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.6

[a] 8-Chloro-11-(piperazin-1-yl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine.

[b] 1-[2-[(2-Amino-4-chlorophenyl)amino]benzoyl]-4-methylpiperazine.

[c] 8-Chloro-5,10-dihydro-11*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one.

[d] 1,4-Bis(8-chloro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-yl)piperazine.

SPECIFIC TESTS

• **Loss on Drying ⟨731⟩**

Sample: Dry at 105° for 4h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards ⟨11⟩**

USP Clozapine RS

USP Clozapine Resolution Mixture RS

Contains a mixture of the following 5 compounds:
Clozapine.
Chlorodibenzodiazepinone;
8-Chloro-5,10-dihydro-11*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one.
$C_{13}H_9ClN_2O$ 244.68
Didiazepinyl piperazine;
1,4-Bis(8-chloro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-yl)piperazine.
$C_{30}H_{24}Cl_2N_6$ 539.46
Demethyl clozapine;

8-Chloro-11-(piperazin-1-yl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine.

$C_{17}H_{17}ClN_4$ 312.80

Benzoyl methylpiperazine analog;
1-[2-[(2-Amino-4-chlorophenyl)amino]benzoyl]-4-methylpiperazine.

$C_{18}H_{21}ClN_4O$ 344.84

[NOTE—The contents have previously been referred to as clozapine, Impurity A, Impurity B, Impurity C, and Impurity D, respectively.]

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
CLOZAPINE	Documentary Standards Support	SM42020 Small Molecules 4

Chromatographic Database Information: Chromatographic Database

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