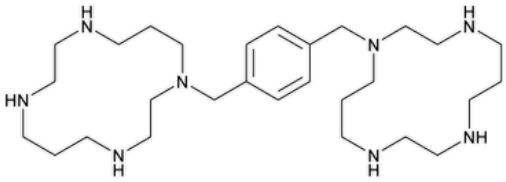
Status: Currently Official on 16-Feb-2025
Official Date: Official as of 01-Aug-2024
Document Type: USP Monographs
DocId: GUID-28B5FFD5-33DE-407B-931A-1641B93E9ABC_2_en-US
DOI: https://doi.org/10.31003/USPNF_M4256_02_01
DOI Ref: pyc6b

Add the following:

▲Plerixafor



$C_{28}H_{54}N_8$ 502.80

1,4,8,11-Tetraazacyclotetradecane, 1,1'-[1,4-phenylenebis(methylene)]bis-;

1,4-Bis[(1,4,8,11-tetraazacyclotetradecan-1-yl)methyl]benzene CAS RN®: 110078-46-1; UNII: S915P5499N.

DEFINITION

Plerixafor contains NLT 97.0% and NMT 103.0% of plerixafor ($C_{28}H_{54}N_8$), calculated on the anhydrous basis.

IDENTIFICATION

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

Solution A: 0.1% (v/v) trifluoroacetic acid in water

Solution B: Methanol and water (50:50). To each liter of the solution, add 1 mL of trifluoroacetic acid.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	80	20	0.8
15	80	20	0.8
41	47	53	0.8
43	47	53	0.8
44	10	90	0.8
45	10	90	1.0
46	80	20	1.0
50	80	20	0.8

Diluent: 0.05 N hydrochloric acid

Standard solution: 1 mg/mL of USP Plerixafor RS in *Diluent*. Sonicate to dissolve.

Sample solution: 1 mg/mL of Plerixafor in *Diluent*. Sonicate to dissolve.

Chromatographic system

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

Mode: LC

Detector: UV 230 nm

Columns

Guard: 4.6-mm × 12.5-mm; 5-µm packing L7

Analytical: 4.6-mm × 15-cm; 3.5-µm packing L7

Column temperature: 40°

Flow rate: See *Table 1*.

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 5.0

Relative standard deviation: NMT 1.10%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of plerixafor ($C_{28}H_{54}N_8$) in the portion of Plerixafor taken:

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

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

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

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

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

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.1%
- **Organic Impurities**

Solution A, Solution B, Mobile phase, Diluent, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 1 mg/mL of USP Plerixafor System Suitability Mixture RS in *Diluent*. Sonicate to dissolve.

Sensitivity solution: 0.0005 mg/mL of USP Plerixafor RS in *Diluent* from the *Standard solution*

System suitability

[Note—Column conditioning may be performed as needed to meet the system suitability requirements using 1.6 mg/mL of USP Plerixafor System Suitability Mixture RS in water as a column conditioning solution. Flush the column with 0.1% v/v trifluoroacetic acid in methanol for 1 h. Equilibrate the column with the starting *Mobile phase* gradient for 30 min. Carry out multiple injections of the column conditioning solution until the resolution criterion of NLT 1.0 between plerixafor 4-benzyl analog and plerixafor 8-benzyl analog is met.]

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

[Note—The relative retention times in *Table 2* are provided as information that could aid in peak assignment.]

Table 2

Name	Relative Retention Time
Plerixafor benzyl alcohol[a]	0.85
Plerixafor	1.00

Name	Relative Retention Time
Plerixafor 4-benzyl analog[b]	2.54
Plerixafor 8-benzyl analog[c]	2.58
Plerixafor 11-benzyl analog[d]	2.90

[a] {4-[(1,4,8,11-Tetraazacyclotetradecan-1-yl)methyl]phenyl}methanol.

[b] 1,4-Bis{4-[(1,4,8,11-tetraazacyclotetradecan-1-yl)methyl]benzyl}-1,4,8,11-tetraazacyclotetradecane.

[c] 1,8-Bis{4-[(1,4,8,11-tetraazacyclotetradecan-1-yl)methyl]benzyl}-1,4,8,11-tetraazacyclotetradecane.

[d] 1,11-Bis{4-[(1,4,8,11-tetraazacyclotetradecan-1-yl)methyl]benzyl}-1,4,8,11-tetraazacyclotetradecane.

Suitability requirements

Resolution: NLT 1.0 between plerixafor 4-benzyl analog and plerixafor 8-benzyl analog; NLT 1.0 between plerixafor and plerixafor benzyl alcohol, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified and unspecified impurity in the portion of Plerixafor taken:

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

r_U = peak response of each specified or unspecified impurity from the *Sample solution*

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

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

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

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. The reporting threshold is 0.05%.

Table 3

Name	Relative Response Factor	Acceptance Criteria, NMT (%)
Plerixafor benzyl alcohol	0.77	0.15
Plerixafor 4-benzyl analog	1.0	0.26
Plerixafor 8-benzyl analog	1.2	0.34
Plerixafor 11-benzyl analog	1.1	0.12
Any unspecified impurity	1.0	0.10
Total impurities[a]	—	1.2

[a] Sum of the results obtained in the *Organic Impurities* and *Limit of Cyclam* tests.

• **Limit of Cyclam**

Solution A: 0.1% (v/v) trifluoroacetic acid in water

Solution B: 0.1% (v/v) trifluoroacetic acid in acetonitrile

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	95	5
15	60	40
20	95	5
30	95	5

Derivatization solution: 20 mg/mL of cupric acetate monohydrate in water

Standard solution: 0.005 mg/mL of USP Cyclam RS in water

Sample solution: 1 mg/mL of Plerixafor in water

Derivatized standard solution: 0.0025 mg/mL of cyclam prepared as follows. Mix 2 mL of the *Standard solution* with 2 mL of the *Derivatization solution* in a suitable head space vial. Crimp to seal the vial and place the vial in a 55° water bath for 15 min. Cool the vial and use for analysis.

Derivatized sample solution: 0.5 mg/mL of plerixafor prepared as follows. Mix 2 mL of the *Sample solution* with 2 mL of the *Derivatization solution* in a suitable head space vial. Crimp to seal the vial and place the vial in a 55° water bath for 15 min. Cool the vial and use for analysis.

Chromatographic system

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

Mode: LC

Detector: UV 266 nm

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

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Derivatized standard solution*

Suitability requirements

Relative standard deviation: NMT 10.0%

Analysis

Samples: *Derivatized standard solution* and *Derivatized sample solution*

Calculate the percentage of cyclam in the portion of Plerixafor taken:

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

r_U = peak response of cyclam from the *Derivatized sample solution*

r_S = peak response of cyclam from the *Derivatized standard solution*

C_S = concentration of USP Cyclam RS in the *Derivatized standard solution* (mg/mL)

C_U = concentration of Plerixafor in the *Derivatized sample solution* (mg/mL)

Acceptance criteria: NMT 0.10%

SPECIFIC TESTS

• **Water Determination ⟨921⟩, *Method I, Method Ic*:** NMT 1.5%

• **Bacterial Endotoxins Test ⟨85⟩:** Where the label states that Plerixafor is sterile or must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Plerixafor is used can be met.

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total aerobic microbial count does not exceed 10^2 cfu/g. The total yeasts and molds count does not exceed 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers at controlled room temperature.

• **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP Reference Standards ⟨11⟩**

USP Cyclam RS

1,4,8,11-Tetraazacyclotetradecane.

$C_{10}H_{24}N_4$ 200.33

USP Plerixafor RS

USP Plerixafor System Suitability Mixture RS

Contains a mixture of the following 5 compounds:

Plerixafor.
Plerixafor 4-benzyl analog: 1,4-Bis{4-[(1,4,8,11-tetraazacyclotetradecan-1-yl)methyl]benzyl}-1,4,8,11-tetraazacyclotetradecane.
Plerixafor 8-benzyl analog: 1,8-Bis{4-[(1,4,8,11-tetraazacyclotetradecan-1-yl)methyl]benzyl}-1,4,8,11-tetraazacyclotetradecane.
Plerixafor 11-benzyl analog: 1,11-Bis{4-[(1,4,8,11-tetraazacyclotetradecan-1-yl)methyl]benzyl}-1,4,8,11-tetraazacyclotetradecane.
Plerixafor benzyl alcohol: {4-[(1,4,8,11-Tetraazacyclotetradecan-1-yl)methyl]phenyl}methanol.

▲ (USP 1-Aug-2024)

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

Topic/Question	Contact	Expert Committee
PLERIXAFOR	Documentary Standards Support	SM22020 Small Molecules 2
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM22020 Small Molecules 2

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. 48(6)

Current DocID: GUID-28B5FFD5-33DE-407B-931A-1641B93E9ABC_2_en-US

DOI: https://doi.org/10.31003/USPNF_M4256_02_01

DOI ref: pyc6b