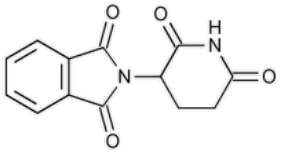
Status: Currently Official on 16-Feb-2025
Official Date: Official as of 01-Dec-2020
Document Type: USP Monographs
DocId: GUID-D0EB84E5-56F9-4F67-ABFE-32E90BA585C2_6_en-US
DOI: https://doi.org/10.31003/USPNF_M82060_06_01
DOI Ref: a6lut

Thalidomide



$C_{13}H_{10}N_2O_4$ 258.23

1*H*-Isoindole-1,3(2*H*)-dione, 2-(2,6-dioxo-3-piperidinyl)-, (±)-;

(±)-*N*-(2,6-Dioxo-3-piperidyl)phthalimide;

α-(*N*-Phthalimido)glutarimide CAS RN®: 50-35-1; UNII: 4Z8R6ORS6L.

DEFINITION

Thalidomide contains NLT 98.0% and NMT 101.5% of thalidomide ($C_{13}H_{10}N_2O_4$), calculated on the anhydrous basis.

IDENTIFICATION

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

Add the following:

▲• **B.** The retention time of the thalidomide peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2020)

ASSAY

Change to read:

• **Procedure**

Solution A: Transfer 1 mL of phosphoric acid into a 100-mL volumetric flask and dilute with water to volume.

Mobile phase: Acetonitrile, water, and phosphoric acid (15: 85: 0.1)

Internal standard solution: 1.5 mg/mL of phenacetin in acetonitrile

Standard stock solution: 1 mg/mL of USP Thalidomide RS in acetonitrile. Sonicate to dissolve, if necessary.

Standard solution: 0.1 mg/mL of USP Thalidomide RS and 0.075 mg/mL of phenacetin prepared as follows. Transfer 10.0 mL of *Standard stock solution* and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, add 10.0 mL of *Solution A*, and dilute with water to volume.

Sample stock solution: 1 mg/mL of Thalidomide in acetonitrile. Sonicate to dissolve, if necessary.

Sample solution: 0.1 mg/mL of Thalidomide and 0.075 mg/mL of phenacetin prepared as follows. Transfer 10.0 mL of *Sample stock solution* and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, add 10.0 mL of *Solution A*, and dilute with water to volume.

Chromatographic system

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

Mode: LC

Detector: UV 237 nm

Column: 3.9-mm × 15-cm; 4-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between thalidomide and phenacetin

▲▲ (USP 1-Dec-2020)

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0% for the response ratio of thalidomide to phenacetin

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of thalidomide ($C_{13}H_{10}N_2O_4$) in the portion of Thalidomide taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of thalidomide to phenacetin from the *Sample solution*

R_S = peak response ratio of thalidomide to phenacetin from the *Standard solution*

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

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

Acceptance criteria: 98.0%–101.5% on the anhydrous basis

IMPURITIES

• **Organic Impurities**

Solution A: Acetonitrile, water, and phosphoric acid (5: 95: 0.1)

Solution B: Acetonitrile, water, and phosphoric acid (15: 85: 0.1)

Solution C: Acetonitrile and water (80:5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	50	50
20	100	0
30	100	0

Diluent A: Acetonitrile, water, and phosphoric acid (50: 50: 0.1)

Diluent B: Transfer 1 mL of phosphoric acid into a 100-mL volumetric flask and dilute with water to volume.

Standard stock solution A: 1 mg/mL of phthalic acid prepared as follows. Transfer 100 mg of phthalic acid to a 100-mL volumetric flask, dissolve in 85 mL of *Solution C*, and dilute with acetonitrile to volume.

Standard stock solution B: 0.1 mg/mL of phthalic acid prepared as follows. Transfer *Standard stock solution A* to a suitable volumetric flask and dilute with acetonitrile to volume.

Standard stock solution C: 1 mg/mL of USP Thalidomide RS in acetonitrile. Sonicate to dissolve, if necessary.

Standard stock solution D: 0.002 mg/mL of phthalic acid and 0.02 mg/mL of USP Thalidomide RS prepared as follows. Pipet 2.0 mL of *Standard stock solution B* and 2.0 mL of *Standard stock solution C* into a 100-mL volumetric flask, and dilute with *Diluent A* to volume.

Standard solution: 0.0002 mg/mL of phthalic acid and 0.002 mg/mL of USP Thalidomide RS prepared as follows. Pipet 10.0 mL of *Standard stock solution D* into a 100-mL volumetric flask, add 10.0 mL of *Diluent B*, and dilute with water to volume.

Sample stock solution: 2 mg/mL of Thalidomide in *Diluent A*. Sonicate to dissolve, if necessary.

Sample solution: 0.2 mg/mL of Thalidomide prepared as follows. Pipet 10.0 mL of *Sample stock solution* into a 100-mL volumetric flask, add 10.0 mL of *Diluent B*, and dilute with water to volume.

Chromatographic system

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

Mode: LC

Detector: UV 218 nm

Column: 3.9-mm × 15-cm; 4-µm packing L1

Flow rate: 2 mL/min

Injection volume: 200 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for phthalic acid and thalidomide are about 0.35 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.0 for phthalic acid and thalidomide

Relative standard deviation: NMT 2.0% for phthalic acid

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Thalidomide taken:

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

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

r_S = peak response of phthalic acid from the *Standard solution*

C_S = concentration of phthalic acid in the *Standard solution* (mg/mL)

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

Acceptance criteria

Individual impurities: NMT 0.1%

Total impurities: NMT 0.3%

Change to read:

• **▲LIMIT OF GLUTAMINE (SEE▲** (USP 1-DEC-2020) **ORDINARY IMPURITIES ⟨466⟩)**

Sample solution: 2 mg/mL of Thalidomide in acetonitrile

Diluent: Acetonitrile and water (1:1)

Standard solution: 0.1 mg/mL of glutamine in *Diluent*

Eluant: Methylene chloride, methanol, and acetic acid (75: 25: 0.05)

Application volume: 2 µL for *Standard solution*; 100 µL for *Sample solution*

Visualization: 4

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

Change to read:

• **MICROBIAL ENUMERATION TESTS ⟨61⟩** and **TESTS FOR SPECIFIED MICROORGANISMS ⟨62⟩:** The total aerobic microbial count ▲▲ (USP 1-Dec-2020) is NMT 10^3 cfu/g, and the total combined molds and yeasts count is NMT 10^2 cfu/g.

• **WATER DETERMINATION ⟨921⟩,** *Method I, Method Ic*

Solvent: Anhydrous dimethyl sulfoxide

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and store at controlled room temperature.

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Thalidomide RS

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

Topic/Question	Contact	Expert Committee
THALIDOMIDE	Documentary Standards Support	SM32020 Small Molecules 3
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. 45(5)

Current DocID: GUID-D0EB84E5-56F9-4F67-ABFE-32E90BA585C2_6_en-US

DOI: https://doi.org/10.31003/USPNF_M82060_06_01

DOI ref: a6lut