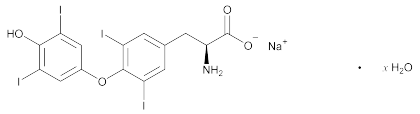
Levothyroxine Sodium



$C_{15}H_{10}I_4NNaO_4 \cdot xH_2O$ (anhydrous) 798.85
L-Tyrosine, *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-, monosodium salt, hydrate;
Monosodium L-thyroxine hydrate [25416-65-3].
Anhydrous [55-03-8].

DEFINITION

Levothyroxine Sodium is the sodium salt of L-3,3′,5,5′-tetraiodothyronine. It contains NLT 97.0% and NMT 103.0% of $C_{15}H_{10}I_4NNaO_4$, calculated on the anhydrous basis.

IDENTIFICATION

- **A.**
  Sample: 50 mg
  Analysis: Ignite the *Sample* in a platinum dish over a flame.
  Acceptance criteria: It decomposes and liberates iodine vapors. [NOTE—Cool the residue, and reserve it for use in *Identification* test *D*.]
- **B.**
  Acid sodium chloride solution: Alcohol, 1 N sodium hydroxide, hydrochloric acid, and water (25:10:10:30)
  Sample: 0.5 mg
  Analysis: Add 7.5 mL of *Acid sodium chloride solution* and 1 mL of 10 mg/mL sodium nitrite solution to the *Sample*. Allow to stand in the dark for 20 min, and add 1.25 mL of ammonium hydroxide.
  Acceptance criteria: A pink color is produced.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay*.
- **D. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨**191**⟩**:** The solution meets the requirements of the flame test.
  Sample solution: To the residue retained from *Identification* test *A,* add a 1 N potassium hydroxide solution dropwise until the residue is dissolved.

ASSAY

- **PROCEDURE**
  Mobile phase: Acetonitrile and water (4:6) that contains 0.5 mL of phosphoric acid in each 1000 mL
  Solution A: 400 mg of sodium hydroxide in 500 mL of water. Cool and add 500 mL of methanol.
  Levothyroxine stock solution: 0.4 mg/mL of USP Levothyroxine RS in *Solution A*
  Liothyronine stock solution: 0.4 mg/mL of liothyronine from USP Liothyronine RS in *Solution A*. Make a 1:100 dilution of this solution using *Mobile phase*.
  Standard solution: 10 µg/mL of levothyroxine from *Levothyroxine stock solution* and 0.2 µg/mL of liothyronine from *Liothyronine stock solution*, in *Mobile phase*
  Sample solution: Prepare a solution of Levothyroxine Sodium in *Mobile phase* having a known concentration of 10 µg/mL. [NOTE—A small amount of 0.01 M methanolic sodium hydroxide can be used to facilitate the dissolution of the sample.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 225 nm
  Column: 4.6-mm × 25-cm; packing L10
  Flow rate: 1.5 mL/min
  Injection size: 100 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 5.0 between liothyronine and levothyroxine
  Relative standard deviation: NMT 2.0% of levothyroxine
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

  r_U = peak response from the *Sample solution*
  r_S = peak response of levothyroxine from the *Standard solution*
  C_S = concentration of USP Levothyroxine RS in the *Standard solution* (µg/mL)
  C_U = concentration of Levothyroxine Sodium in the *Sample solution* (µg/mL)
  M_{r1} = molecular weight of levothyroxine sodium, 798.85
  M_{r2} = molecular weight of levothyroxine, 776.87
  Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

Change to read:

■[NOTE—On the basis of the synthetic route, perform either *Organic Impurities, Procedure 1* or *Procedure 2*. *Procedure 2* is recommended when related compounds listed in *Table 3* may be present.]■1S *(USP33)*

- **ORGANIC IMPURITIES,** *Procedure 1*
  Diluent: Acetonitrile and water (1:1)
  Solution A: Dilute 5 mL of phosphoric acid with *Diluent* to 100.0 mL.
  Mobile phase: Dissolve 1.0 g of sodium 1-heptanesulfonate in 200 mL of water. Add 200 mL of acetonitrile, 400 mL of methanol, and 1.0 mL of phosphoric acid. Dilute with water to 1 L.
  Standard stock solution 1: Transfer 25 mg of USP Levothyroxine RS to a 100-mL volumetric flask. Add 50 mL of *Diluent* and 1 drop of 10 N sodium hydroxide, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.
  Standard stock solution 2: Transfer 25 mg of USP Liothyronine RS to a 100-mL volumetric flask. Add 50 mL of *Diluent* and 1 drop of 10 N sodium hydroxide, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.
  System suitability solution: Transfer 5.0 mL of *Standard stock solution 1* and 5.0 mL of *Standard stock solution 2* to a 100-mL volumetric flask. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.
  Standard solution: Pipet 4.0 mL of the *System suitability solution* to a 100-mL volumetric flask. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.
  Blank solution: Add 7 mL of *Solution A* to a 100-mL volumetric flask, and dilute with *Diluent* to volume.
  Sample solution: Transfer 25 mg of Levothyroxine Sodium to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and

sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection size: 15 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between levothyroxine and liothyronine, *System suitability solution*
Relative standard deviation: NMT 2.0% for the levothyroxine peak, *Standard solution*

Analysis
Samples: *Standard solution*, *Blank solution*, and *Sample solution*
[NOTE—Record the chromatograms for at least six times the retention time of the levothyroxine peak. Verify that no peaks elute in the *Blank solution* at the expected retention times for levothyroxine and related compounds.]
Calculate the area percentage of each related compound in the portion of Levothyroxine ■Sodium■1S (*USP33*) taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$ ■1S (*USP33*)

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of levothyroxine from the *Standard solution*
C_S = concentration of levothyroxine in the *Standard solution* (mg/mL)
C_U = concentration of Levothyroxine Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of levothyroxine sodium, 798.85
M_{r2} = molecular weight of levothyroxine, 776.87
■■1S (*USP33*)

[NOTE—The relative response factor for the impurities listed in *Table 1* is 1.00. Any unspecified impurity peaks should be assigned a relative response factor of 1.00.]
Disregard peaks corresponding to those of the *Blank solution*, and disregard peaks corresponding to less than 0.03%.
Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Liothyronine	0.65–0.70	1.0
β-Hydroxy-T4[a]	0.71–0.76	0.15
Levothyroxine	1.0	—
T4-Hydroxyacetic acid[b]	1.13–1.28	0.15
N-Formyl-T4[c] and T4-acetamide[d]	1.47–1.53	0.15
N-Acetyl-T4[e]	1.50–1.86	0.20
T4-Acetic acid[f]	2.42–2.51	■0.30■1S (*USP33*)
T4-Aldehyde[g]	3.17–3.45	0.15
T4-Benzoic acid[h]	3.46–3.70	0.15
Individual unspecified impurity	—	0.10
Total impurities	—	■2.0■1S (*USP33*)

[a] *O*-(4-Hydroxy-3,5-diiodophenyl)-3,5-diiodo-β-hydroxy-L-tyrosine.
[b] 2-Hydroxy-2-(4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetic acid.
[c] *N*-Formyl-*O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.
[d] 2-(4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl) acetamide.
[e] *N*-Acetyl-*O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.
[f] 2-(4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetic acid.
[g] 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzaldehyde.
[h] 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzoic acid.

Add the following:

■• **ORGANIC IMPURITIES,** *Procedure 2*
Solution A: Dissolve 9.7 g of sulfamic acid in 2000 mL of water. Add 1.5 g of sodium hydroxide, mix to dissolve, and adjust with 2 N sodium hydroxide to a pH of 2.0.
Solution B: Acetonitrile
Diluent 1: Methanol and *Solution A* (90:10)
Diluent 2: Acetonitrile and *Solution A* (30:70); mix with *Diluent 1* (1:1).
Mobile phase: See *Table 2* below.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	70	30
10	70	30
40	20	80
50	20	80
53	70	30
75	70	30

Blank solution: Use *Diluent 2*.
Standard stock solution: 0.1 mg/mL of USP Levothyroxine RS and USP Liothyronine RS in *Diluent 1*
Standard solution: 0.002 mg/mL of USP Levothyroxine RS and USP Liothyronine RS, prepared using the *Standard stock solution* in *Diluent 2*
Sensitivity solution: 0.0002 mg/mL of USP Levothyroxine RS and USP Liothyronine RS, prepared using the *Standard solution* in *Diluent 2*
Identification solution: Dissolve 5.0 mg of USP Levothyroxine for Peak Identification RS in 4.5 mL of methanol. Add 0.5 mL of *Solution A*. Further dilute a portion of this solution with *Diluent 2* to obtain a solution containing about 0.2 mg/mL.
Sample solution: Dissolve an amount of Levothyroxine Sodium in *Diluent 1* to obtain a solution having a known concentration of about 1.0 mg/mL. Further dilute a portion of this solution with *Diluent 2* to obtain a solution having a known concentration of about 0.2 mg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC
Detector: UV 225 nm
Column: 4.0-mm × 15-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection size: 25 µL

System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 5 between levothyroxine and liothyronine, *Standard solution*
Signal-to-noise ratio: NLT 5 for each peak from the *Sensitivity solution*, calculated by:

$$\text{Result} = (2H)/h$$

H = measured height of the peak
h = amplitude of the average measured baseline noise

Analysis
Samples: *Blank solution*, *Standard solution*, *Identification solution*, and *Sample solution*
[NOTE—Identify the components on the basis of their relative retention times as listed in *Table 3*.]
Calculate the percentage of liothyronine sodium in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of liothyronine from the *Sample solution*
r_S = peak response of liothyronine from the *Standard solution*
C_S = concentration of liothyronine in the *Standard solution* (mg/mL)
C_U = concentration of Levothyroxine Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of liothyronine sodium, 672.96
M_{r2} = molecular weight of liothyronine, 650.98

Calculate the percentage of any other impurity in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of levothyroxine from the *Standard solution*
C_S = concentration of levothyroxine in the *Standard solution* (mg/mL)
C_U = concentration of Levothyroxine Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of levothyroxine sodium, 798.85
M_{r2} = molecular weight of levothyroxine, 776.87

[NOTE—The relative response factor for the impurities listed in *Table 3* is 1.00. Any unspecified impurity peaks should be assigned a relative response factor of 1.00.]
Disregard peaks corresponding to those of the *Blank solution*, and disregard peaks corresponding to less than 0.03%.

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Liothyronine	0.65	1.0
Monochlorotriiodothyronine[a]	0.94	0.15
●Levothyroxine *N*-methylamide[b]	0.97	0.15● (RB 1-Oct-2010)
Levothyroxine	1.0	—
Triiodothyroacetic acid, or T3-acetic acid[c]	1.57	0.15
O-(4-Hydroxy-3,5-diiodophenyl)thyroxine, or T6[d]	1.61	0.50
O-Methyl-tetraiodothyroethylamine, or T4-amine *O*-methyl[e]	1.76	0.30
T4-Acetic acid[f]	1.79	0.30
Individual unspecified impurity	—	0.10
Total impurities	—	2.0

[a] (*S*)-2-Amino-3-[3-chloro-4-(4-hydroxy-3,5-diiodophenoxy)-5-iodophenyl]propanoic acid.
●[b] (*S*)-2-Amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]-*N*-methylpropanamide.● (RB 1-Oct-2010)
[c] [4-(4-Hydroxy-3-iodophenoxy)-3,5-diiodophenyl]acetic acid.
[d] (*S*)-2-Amino-3-[4-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenoxy]-3,5-diiodophenyl]propanoic acid.
[e] 2-[4-(3,5-Diiodo-4-methoxyphenoxy)-3,5-diiodophenyl]ethanamine.
[f] 2-(4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetic acid.

■1S (*USP33*)

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** –5° to –6°
  Sample solution: Equivalent to 30 mg/mL of anhydrous Levothyroxine Sodium, in alcohol and 1 N sodium hydroxide (2:1)
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 11.0%

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. ■Store as stated in the labeling instructions.■1S (*USP33*)

Add the following:

■● **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.■1S (*USP33*)

Change to read:

- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Levothyroxine RS
  O-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.
  $C_{15}H_{11}I_4NO_4$ 776.87
  USP Liothyronine RS
  O-(4-hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosine.
  $C_{15}H_{12}I_3NO_4$ 650.98
  ■USP Levothyroxine for Peak Identification RS
  Levothyroxine sodium spiked with liothyronine, triiodothyroacetic acid, tetraiodothyroacetic acid.
  ■1S (*USP33*)