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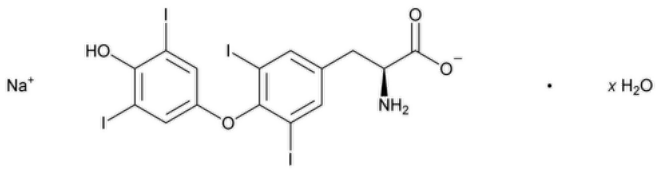
Levothyroxine Sodium



$C_{15}H_{10}I_4NNaO_4 \cdot xH_2O$ (anhydrous) 798.85

L-Tyrosine, *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-, monosodium salt, hydrate;

Monosodium L-thyroxine hydrate CAS RN®: 25416-65-3.

Anhydrous CAS RN®: 55-03-8; UNII: 054I36CPMN.

DEFINITION

Levothyroxine Sodium is the sodium salt of L-3,3',5,5'-tetraiodothyronine. It contains NLT 97.0% and NMT 103.0% of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$), 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*.
- **C. Identification Tests—General ⟨191⟩, *Chemical Identification Tests, Sodium***

Sample solution: To 200 mg add 2 mL of 2 N sulfuric acid. Heat on a water bath and then carefully heat over an open flame, increasing the temperature gradually up to about 600°. [Note—Alternative procedures for igniting the material could also be used.] Continue the ignition until most of the particles have disappeared. Dissolve the residue in 2 mL of water.

Acceptance criteria: The *Sample solution* meets the requirements of test *A*.

ASSAY

Change to read:

- **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 ▲▲ (USP 1-Aug-2020) USP Liothyronine RS in *Solution A*. ▲Further dilute with *Mobile phase* to 0.004 mg/mL.▲ (USP 1-Aug-2020)

Standard solution: 10 µg/mL of ▲USP Levothyroxine RS▲ (USP 1-Aug-2020) from *Levothyroxine stock solution* and 0.2 µg/mL of ▲USP Liothyronine RS▲ (USP 1-Aug-2020) from *Liothyronine stock solution* in *Mobile phase*

Sample solution: 10 µg/mL of Levothyroxine Sodium in *Mobile phase*. [Note—A small amount of 0.01 M sodium hydroxide▲ in methanol▲ (USP 1-Aug-2020) 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; ▲5-µm▲ (USP 1-Aug-2020) packing L10

Flow rate: 1.5 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between liothyronine and levothyroxine

Relative standard deviation: NMT 2.0% for 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 of levothyroxine 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

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

Change to read:

• **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: ▲1.0 g/L of▲ (USP 1-Aug-2020) sodium 1-heptanesulfonate▲prepared as follows. Transfer 1 g of sodium 1-heptanesulfonate to a 1-L volumetric flask and dissolve▲ (USP 1-Aug-2020) 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 ▲volume.

Levothyroxine stock solution: 0.25 mg/mL of USP Levothyroxine RS prepared as follows.▲ (USP 1-Aug-2020) Transfer 25 mg of USP Levothyroxine RS to a 100-mL volumetric flask ▲containing▲ (USP 1-Aug-2020) 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.

▲**Liothyronine stock solution:** 0.25 mg/mL of USP Liothyronine RS prepared as follows.▲ (USP 1-Aug-2020) Transfer 25 mg of USP Liothyronine RS to a 100-mL volumetric flask ▲containing▲ (USP 1-Aug-2020) 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: ▲0.0125 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS prepared as follows.▲ (USP 1-Aug-2020) Transfer 5.0 mL each of ▲*Levothyroxine stock solution*▲ (USP 1-Aug-2020) and ▲*Liothyronine stock solution*▲ (USP 1-Aug-2020) to a 100-mL volumetric flask. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Standard solution: ▲0.0005 mg/mL of USP Levothyroxine RS and USP Liothyronine RS prepared as follows.▲ (USP 1-Aug-2020) Transfer 4.0 mL of *System suitability solution* into a 100-mL volumetric flask. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Sample solution: ▲0.25 mg/mL of Levothyroxine Sodium prepared as follows.▲ (USP 1-Aug-2020) 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.

Blank solution: Transfer 7 mL of *Solution A* to a 100-mL volumetric flask, 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 volume: 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*, *Sample solution*, and *Blank solution*

[Note—Record the chromatograms for at least 6 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 ▲impurity▲ (USP 1-Aug-2020) 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 each impurity 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* (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

▲▲ (USP 1-Aug-2020)

Disregard peaks corresponding to those of the *Blank solution*.▲▲ (USP 1-Aug-2020)

Acceptance criteria: See *Table 1*. ▲The reporting threshold is 0.03%.▲ (USP 1-Aug-2020)

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
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

[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.

Change to read:

• **Organic Impurities, Procedure 2**

Solution A: ▲4.85 g/L of sulfamic acid and 0.75 g/L of sodium hydroxide in water.▲ (USP 1-Aug-2020) Adjust with 2 N sodium hydroxide to a pH of 2.0.

Solution B: Acetonitrile

Mobile phase: See *Table 2*.

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

Diluent 1: Methanol and *Solution A* (90:10)

Diluent 2: Acetonitrile and *Solution A* (30:70). Mix with *Diluent 1* (1:1).

Identification solution: 0.2 mg/mL of USP Levothyroxine Peak Identification Mixture RS in *Diluent 2*

Standard stock solution: 0.1 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS in *Diluent 1*

Standard solution: 0.002 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS, ▲from▲ (USP 1-Aug-2020) *Standard stock solution* in *Diluent 2*

Sensitivity solution: 0.0002 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS, ▲from▲ (USP 1-Aug-2020) *Standard solution* in *Diluent 2*

Sample solution: ▲1.0 mg/mL of Levothyroxine Sodium in *Diluent 1*. Further dilute a portion of this solution with *Diluent 2* to 0.2 mg/mL.▲ (USP 1-Aug-2020)

Blank solution: Use *Diluent 2*.

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 volume: 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, *Sensitivity solution*▲ (USP 1-Aug-2020)

Analysis

Samples: *Identification solution*, *Standard solution*, *Sample solution*, and *Blank 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 USP Liothyronine RS 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, ▲650.97▲ (USP 1-Aug-2020)

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 ▲other▲ (USP 1-Aug-2020) impurity 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* (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

▲▲ (USP 1-Aug-2020)

Disregard peaks corresponding to those of the *Blank solution*. ▲▲ (USP 1-Aug-2020)

Acceptance criteria: See *Table 3*. ▲The reporting threshold is 0.03%.▲ (USP 1-Aug-2020)

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
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
▲T4-Formic acid *N*-methylamide▲ (USP 1-Aug-2020) [e]	1.76	0.30
T4-Acetic acid[f]	1.79	0.30
▲Tetraiodothyrobenzoic acid or T4-benzoic acid[g]	1.95	0.15▲ (USP 1-Aug-2020)
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.

[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] 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodo-*N*-methylbenzamide.▲ (USP 1-Aug-2020)

[f] 2-(4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetic acid.

▲g 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzoic acid.▲ (USP 1-Aug-2020)

SPECIFIC TESTS

- **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*

  Sample solution: Equivalent to 30 mg/mL of anhydrous Levothyroxine Sodium in alcohol and 1 N sodium hydroxide (2:1)

  Acceptance criteria: −5° to −6°

- **Water Determination** ⟨921⟩, *Method I*: NMT 11.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, protected from light. Store as stated in the labeling instructions.
- **Labeling:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.

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 Levothyroxine Peak Identification Mixture RS

Levothyroxine sodium spiked with liothyronine, triiodothyroacetic acid, and tetraiodothyroacetic acid in methanol.

USP Levothyroxine Sodium RS

USP Liothyronine RS

O-(4-Hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosine.

$C_{15}H_{12}I_3NO_4$ ▲650.97▲ (USP 1-Aug-2020)

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

Topic/Question	Contact	Expert Committee
LEVOTHYROXINE SODIUM	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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