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# Thyroid

## DEFINITION

Thyroid is the cleaned, dried, and powdered thyroid gland previously deprived of connective tissue and fat. It is obtained from domesticated animals that are used for food by humans.

On hydrolysis it yields NLT 90.0% and NMT 110.0% each of the labeled amounts of levothyroxine ($C_{15}H_{11}I_4NO_4$) and liothyronine ($C_{15}H_{12}I_3NO_4$), calculated on the dried basis. It is free from iodine in inorganic or any form of combination other than that peculiar to the thyroid gland. It may contain a suitable diluent such as lactose, sodium chloride, starch, sucrose, or dextrose.

## IDENTIFICATION

• **A.** The retention times of the peaks for liothyronine and levothyroxine of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

***Change to read:***

• **Procedure**

**Mobile phase:** Acetonitrile, water, and phosphoric acid (350:650:5), filtered and degassed

**Reducing buffer solution:** Freshly prepare 0.04 M tris(hydroxymethyl)aminomethane and 0.05 M methimazole in 0.11 M sodium chloride. Adjust, if necessary, with 6 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 8.4 ± 0.05.

**Proteolytic enzyme:** Freshly prepare a solution containing 3 mg/mL of bacterial protease in *Reducing buffer solution*.[1]

**Enzyme deactivating solution:** Phosphoric acid in acetonitrile (1:99)

**Standard stock solution:** Transfer accurately weighed quantities of about 9 mg of USP Liothyronine RS and about 38 mg of USP Levothyroxine RS to a 100-mL volumetric flask, add 50 mL of a mixture of acetonitrile, water, and ammonium hydroxide (500:500:1) and swirl to dissolve. Dilute with a mixture of acetonitrile and water (1:1) to volume, and mix. [Note—Protect solutions from light.]

**Standard solution:** Pipet 5 mL of the freshly prepared *Standard stock solution* into a 250-mL volumetric flask, dilute with *Reducing buffer solution* to volume, and mix to obtain a solution having known concentrations of about 1.8 µg/mL of liothyronine and 7.6 µg/mL of levothyroxine. Pipet 5 mL of this solution into a screw-capped 16- × 125-mm culture tube. Pipet 2 mL of *Enzyme deactivating solution* into the tube, place the cap on the tube, and shake the mixture vigorously. [Note—Prepare on the day of use.]

**Sample solution:** Transfer an accurately weighed portion of finely powdered Thyroid, equivalent to about 38 µg of levothyroxine, to a screw-capped 16- × 125-mm culture tube that has been flushed previously with nitrogen. Taking precautions to avoid unnecessary exposure to air, pipet 5 mL of *Proteolytic enzyme* into the tube. Allow nitrogen to flow gently over the mixture for 5 min. Place the cap on the tube, mix to disperse the contents, and place in a covered water bath maintained at a temperature of 37 ± 1° for 28 h. [Note—Protect the contents of the tubes from light.]

Examine occasionally, and mix as necessary to ensure dispersion. At the end of the incubation period, pipet 2 mL of *Enzyme deactivating solution* into the tube, place the cap on the tube, mix vigorously, and centrifuge at about 2000 rpm for 5 min. Pass the supernatant through a filter of 0.45-µm pore size, discarding the first 1 mL of the filtrate.

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 230 nm

**Column:** ▲4.6-mm▲ (ERR 1-Apr-2023) × 25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 200 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.8 for liothyronine and levothyroxine peaks

**Relative standard deviation:** NMT 2.0% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of levothyroxine ($C_{15}H_{11}I_4NO_4$) and liothyronine ($C_{15}H_{12}I_3NO_4$) in the portion of Thyroid taken:

$$\text{Result} = (V \times C) \times (r_U/r_S)$$

$V$ = volume of *Sample solution*, 7 mL

$C$ = concentration of the corresponding Reference Standard in the *Standard solution* (µg/mL)

$r_U$ = peak response of the corresponding analytes from the *Sample solution*

$r_S$ = peak response of the corresponding analytes from the *Standard solution*

**Acceptance criteria:** 90.0%–110.0% each of the labeled amounts of levothyroxine ($C_{15}H_{11}I_4NO_4$) and liothyronine ($C_{15}H_{12}I_3NO_4$) on the dried basis

## IMPURITIES

• **Limit of Inorganic Iodides**

**Extracting solution:** Sulfuric acid and water (1:99)

**Standard solution:** Dissolve an accurately weighed quantity of potassium iodide in water to obtain a stock solution containing 0.131 mg, equivalent to 0.100 mg of iodide, per mL. Transfer 1.0 mL of this stock solution into a 100-mL volumetric flask, dilute with *Extracting solution* to volume, and mix. Each mL of the *Standard solution* contains 1.0 µg of iodide. [Note—Prepare this solution on the day of use.]

**Sample solution:** Transfer 1.00 g, or proportionately less if the dry basis levothyroxine content is greater than 38 µg per grain, of Thyroid to a beaker. Add 100.0 mL of *Extracting solution*, and sonicate for 5 min.

**Electrode system:** Use an iodide-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±1 mV (see *pH* ⟨791⟩).

**Analysis:** Transfer the *Standard solution* to a beaker containing a magnetic stirring bar. Rinse and dry the electrodes, insert in the solution, stir for 5 min or until the reading stabilizes, and read the potential in mV. Repeat this process using the *Sample solution*.

**Acceptance criteria:** The requirements of the test are met if the *Sample solution* has a higher potential, in mV, than the *Standard solution*, the limit is 0.01%.

## SPECIFIC TESTS

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** It meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*.

• **Loss on Drying** ⟨731⟩

**Analysis:** Dry under vacuum at 60° for 4 h.

**Acceptance criteria:** NMT 6.0%

## ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **USP Reference Standards** ⟨11⟩

USP Levothyroxine RS

USP Liothyronine RS

[1] A suitable grade is available as “Pronase” (Catalog number 53702) from Calbiochem-Behring, P.O. Box 12087, San Diego, CA 92112.

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

| Topic/Question | Contact | Expert Committee |
|---|---|---|
| THYROID | Jennifer Tong Sun<br>Senior Scientist II | BIO32020 Biologics Monographs 3 - Complex Biologics and Vaccines |
| REFERENCE STANDARD SUPPORT | RS Technical Services<br>RSTECH@usp.org | BIO32020 Biologics Monographs 3 - Complex Biologics and Vaccines |

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