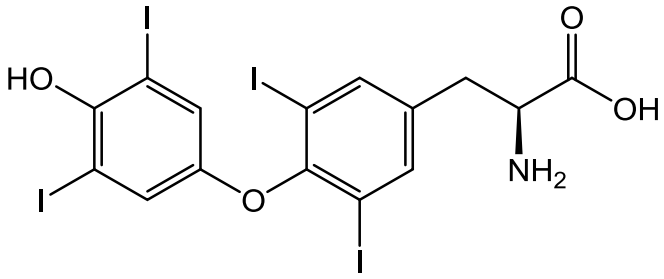
Certificate of Analysis

ISO 17034
ANAB Cert# AR-1470

ISO/IEC 17025
ANAB Cert# AT-1467

LEVOTHYROXINE

CERTIFIED REFERENCE MATERIAL



CERTIFIED PURITY:

95.1%, U_{crm} = ±0.6% k = 2.1 (Mass Balance/dried basis)

94.6%, U_{crm} = ±0.6% k = 2.1 (Mass Balance/as is basis)

NOMINAL PACKAGE SIZE: 1g

CATALOG #: PHR1613 **LOT #:** LRAC1514

CERTIFICATE VERSION: LRAC1514.1 **ISSUE DATE:** 12 June 2019

Note: Certificates may be updated due to Pharmacopeial Lot changes or the availability of new data. Check our website at: www.sigma-aldrich.com *for the most current version.*

CRM EXPIRATION: 30 June 2023 (Proper Storage and Handling Required).

RECEIPT DATE: _____

Note: this space is provided for convenience only and its use is not required.

STORAGE: Store at Room Temperature/Protect from Light, keep container tightly closed. Attachment of a 20 mm aluminum crimp seal recommended for unused portions.

CHEMICAL FORMULA: $C_{15}H_{11}I_4NO_4$ **MW:** 776.9

PHYSICAL DESCRIPTION: White powder in amber vial **CAS #:** 51-48-9

HAZARDS: Read Safety Data Sheet before using. All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel.

INSTRUCTIONS FOR USE: For USP quantitative applications, correct for moisture, determined by drying a separate portion under vacuum at 60˚C for 4 hours. For EP applications, do not dry, use on the as is basis. The internal pressure of the container may be slightly different from the atmospheric pressure at the user's location. Open slowly and carefully to avoid dispersion of the material. This material is intended for Laboratory use only. Not for drug, household or other uses.

TRACEABILITY ASSAY

Comparative assay demonstrates direct traceability to Pharmacopeial Standards

ASSAY vs. USP REFERENCE STANDARD (dried basis)

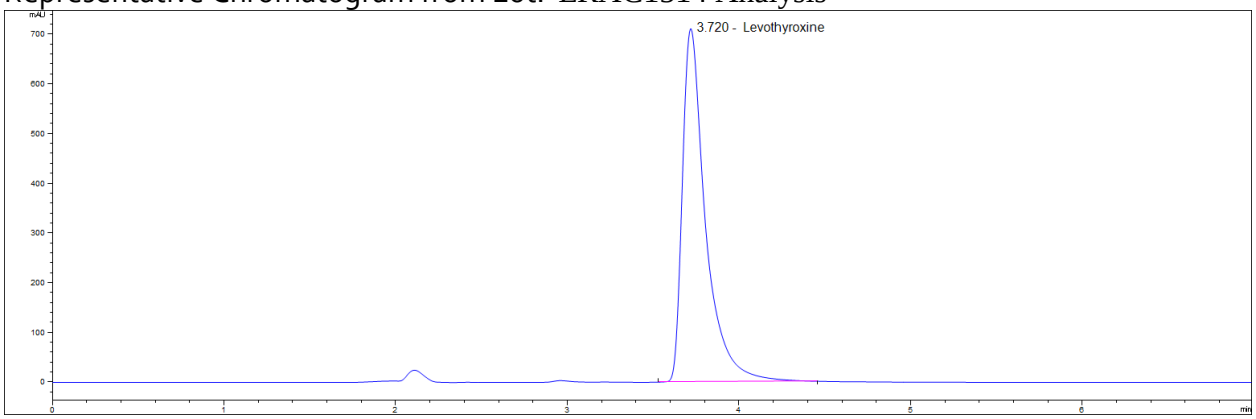
ASSAY VALUE	vs. USP LOT
96.2%	**R016X1**
	Labeled Content = 0.987 mg/mg

ASSAY vs. EP CRS (as is basis)

ASSAY VALUE	vs. EP BATCH
95.3%	**4.0**
	Labeled Content = 89.2%, as $C_{15}H_{10}I_4NNaO_4$

METHOD: HPLC (ref.: Levothyroxine Sodium, Current Compendial Monographs)
Column: Ascentis Express ES-CN, 4.6 x 100mm, 2.7µm
Mobile Phase: 0.05% H_3PO_4 in Water, 0.05% H_3PO_4 in Acetonitrile (60:40)
Flow Rate: 0.5 mL/min
Column Temperature: 30 ºC
Injection: 5 µL
Detector: 225 nm

Representative Chromatogram from Lot: LRAC1514 Analysis



PURITY DETERMINATION BY MASS BALANCE

CHROMATOGRAPHIC IMPURITY ANALYSIS

METHOD: HPLC (ref.: Levothyroxine, Current Compendial Monographs)

Column: Ascentis Express ES-CN, 4.6 x 100mm, 2.7µm
Mobile Phase A: 0.05% H_3PO_4 in Water
Mobile Phase B: 0.05% H_3PO_4 in Acetonitrile
Gradient:

Time (min)	% A	% B
0-8.5	70	30
8.5-35	70-20	30-80
35-44	20	80
44-44.1	20-70	80-30

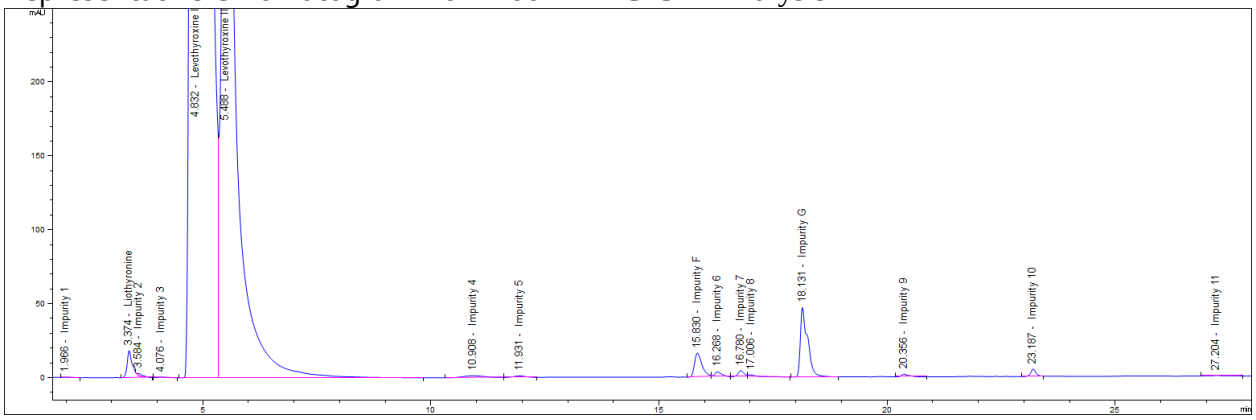
Flow Rate: 1.0 mL/min
Column Temperature: 30 ºC
Injection: 25 µL
Detector: 225 nm

Impurities Detected:

Impurity 1:	0.0055 %	Liothyronine:	0.4058 %
Impurity 2:	0.0463 %	Impurity 3:	0.0111 %
Impurity 4:	0.1066 %	Impurity 5:	0.0649 %
Impurity F:	0.4215 %	Impurity 6:	0.1813 %
Impurity 7:	0.1197 %	Impurity 8:	0.0466 %
Impurity G:	1.3200 %	Impurity 9:	0.540 %
Impurity 10:	0.0987 %	Impurity 11:	0.0141 %

Total Impurities: **2.896 %**

Representative Chromatogram from Lot: LRAC1514 Analysis



RESIDUAL SOLVENTS

Method: GC-MS Headspace (ref.: Adapted from Residual Solvents USP <467>)
Column: SPB-624
Carrier gas: He
Flow: 1.2 mL/min
Split Ratio: 1:5
Injection/Temperature: 1 mL/220 °C
Temperature Program: 40 °C for 5 min, 8 °C/min to 200 °C, hold 5 min

Solvents Detected:

Ethanol: **0.1313 %**

LOSS ON DRYING/VOLATILES

Method: Under vacuum at 60ºC (ref.: Current Compendial Monographs)
Mean of three measurements, Loss = **0.344 %**

RESIDUE ANALYSIS

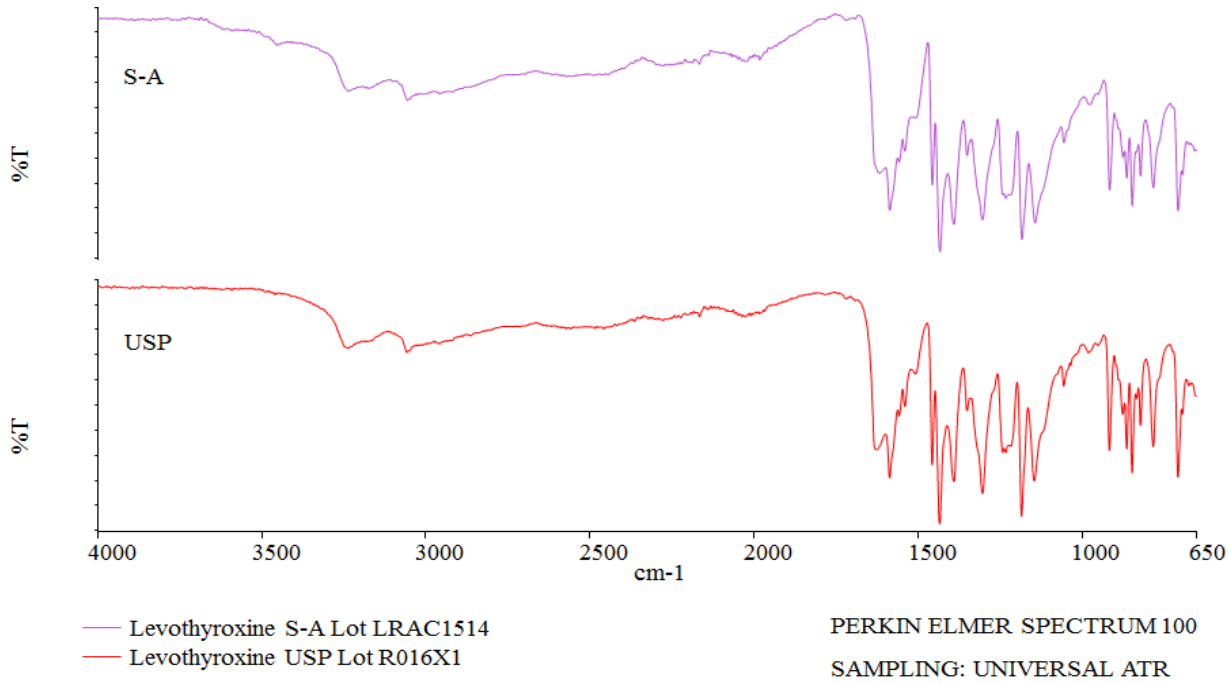
Method: Sulfated Ash (ref.: Current Compendial Monographs)
Sample Size: ~ 90 mg
Mean of three measurements, Residue = **2.109 %**

CERTIFIED PURITY BY MASS BALANCE [100% - Impurities (normalized)]

95.1% U_{crm} = ±0.6%, k = 2.1 (dried basis)
94.6% U_{crm} = ±0.6%, k = 2.1 (as is basis)

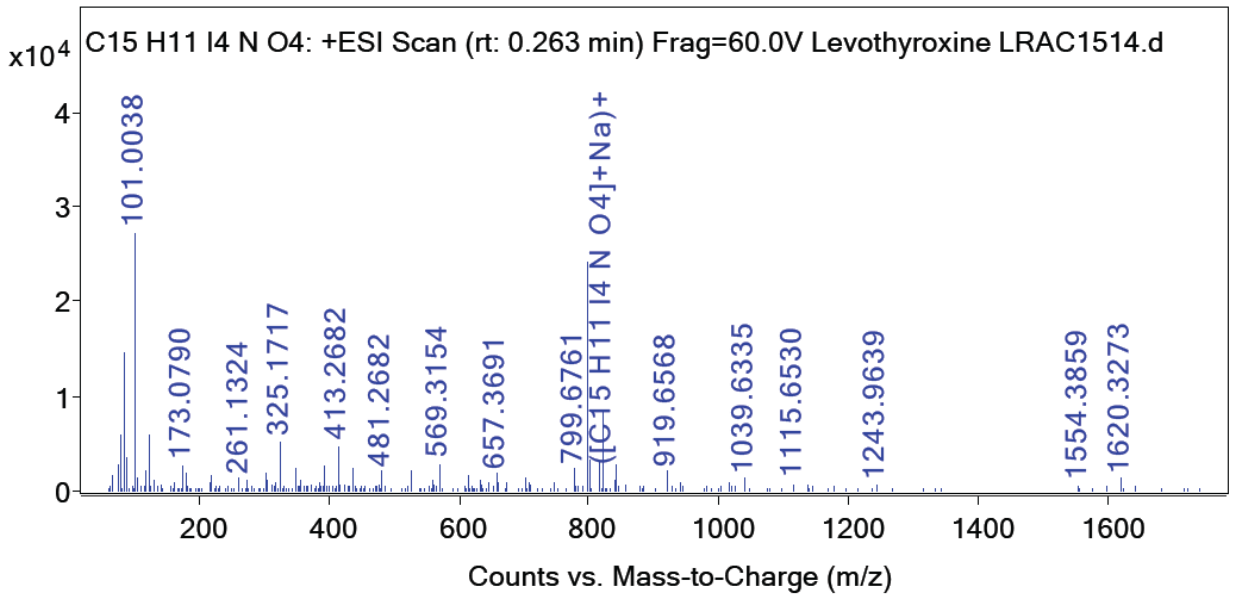
IDENTIFICATION TESTS

INFRARED SPECTROPHOTOMETRY (Comparative identification analysis demonstrates direct traceability to Pharmacopeial standards)



MASS SPECTRUM

Method: HR-QTOF; 4.0 kV ESI+; temperature: 325 °C



Theoretical value: 799.6765 *m/z*

The signal of the MS spectrum is consistent with the theoretical value and its interpretation is consistent with the structural formula.

HOMOGENEITY ASSESSMENT

Homogeneity was assessed in accordance with ISO Guide 35. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared by Single Factor Analysis of Variance (ANOVA). The uncertainty due to homogeneity was derived from the ANOVA. Heterogeneity was not detected under the conditions of the ANOVA.

Analytical Method: HPLC Sample size: ~ 25 mg

UNCERTAINTY STATEMENT

Uncertainty values in this document are expressed as Expanded Uncertainty (U_{crm}) corresponding to the 95% confidence interval. U_{crm} is derived from the combined standard uncertainty multiplied by the coverage factor k, which is obtained from a *t*-distribution and degrees of freedom. The components of combined standard uncertainty include the uncertainties due to characterization, homogeneity, long term stability, and short term stability (transport). The components due to stability are generally considered to be negligible unless otherwise indicated by stability studies.

STABILITY ASSESSMENT

Significance of the stability assessment will be demonstrated if the analytical result of the study and the range of values represented by the Expanded Uncertainty do not overlap the result of the original assay and the range of its values represented by the Expanded Uncertainty. The method employed will usually be the same method used to characterize the assay value in the initial evaluation.

Long Term Stability Evaluation - An assessment, or re-test, versus a Compendial Reference Standard may be scheduled, within the 3 year anniversary date of a release of a Secondary Standard. The re-test interval will be determined on a case-by-case basis.

Short Term Stability Study - It is useful to assess stability under reasonably anticipated, short term transport conditions by simulating exposure of the product to humidity and temperature stress. This type of study is conducted under controlled conditions of elevated temperature and humidity.

QC Manager

Head Quality Assurance

APPENDIX

Original Release Date: 12 June 2019

Manufactured and certified by Sigma-Aldrich RTC, Inc.
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ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

ANAB ACCREDITED ISO 17034 REFERENCE MATERIAL PRODUCER