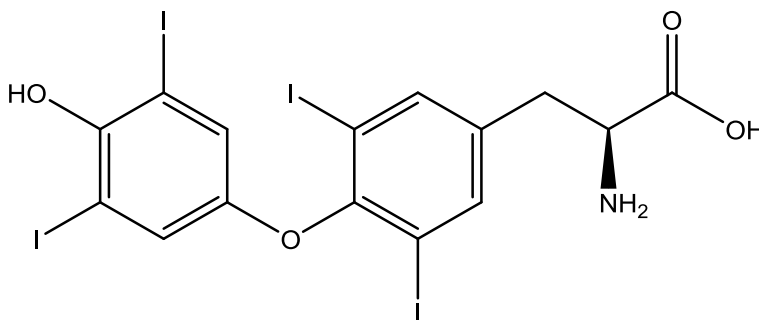


Certificate of Analysis

ISO GUIDE 34
ANAB Cert# AR-1470

ISO/IEC 17025
ANAB Cert# AT-1467

LEVOTHYROXINE CERTIFIED REFERENCE MATERIAL



CERTIFIED PURITY:

97.0%, $U_{\text{crm}} = \pm 0.1\%$ $k = 2$ (Mass Balance/dried basis)

96.6%, $U_{\text{crm}} = \pm 0.5\%$ $k = 4.30$ (Mass Balance/as is basis)

NOMINAL PACKAGE SIZE: 1g

CATALOG #: PHR1613

LOT #: LRAA4872

CERTIFICATE VERSION: LRAA4872.2

ISSUE DATE: 31 March 2016

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: 31 December 2019 (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.

SIGMA-ALDRICH®

INSTRUCTIONS FOR USE: For USP applications, dry portion under vacuum at 60°C for 4 hours prior to use and correct for moisture. 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 R&D use only. Not for drug, household or other uses.

TRACEABILITY ASSAY

Comparative assay demonstrates direct traceability to Pharmacopeial Standards
Specification: 97.0-103.0% (USP)

ASSAY vs. USP REFERENCE STANDARD (dried basis)

ASSAY VALUE

97.7%

vs. USP LOT

R016X1

Labeled Content = 0.987mg/mg

METHOD: HPLC (ref.: Levothyroxine Sodium, USP38)

Column: Ascentis Express ES-CN, 4.6 x 100mm, 2.7µm

Mobile Phase: 0.05% H₃PO₄ in Water, 0.05% H₃PO₄ in Acetonitrile (60:40)

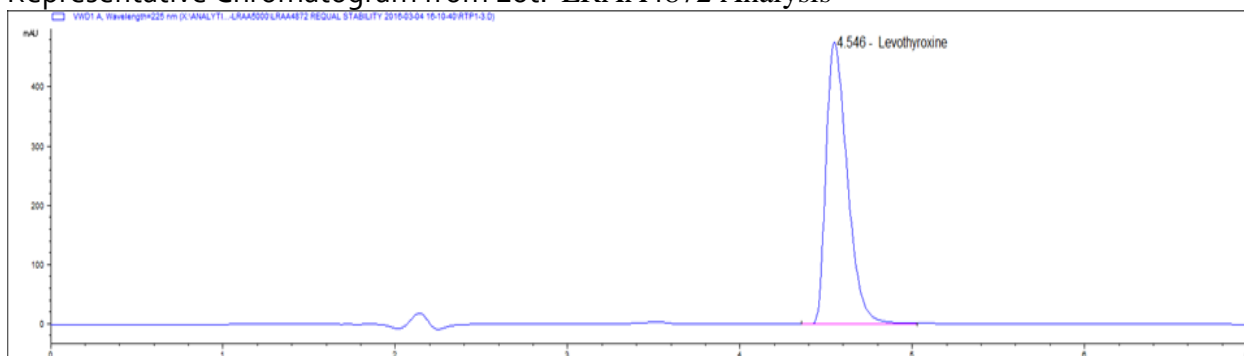
Flow Rate: 0.5mL/min

Column Temperature: 30°C

Injection: 6µL

Detector: 225nm

Representative Chromatogram from Lot: LRAA4872 Analysis



ASSAY vs. EP CRS (as is basis)

ASSAY VALUE

95.4%

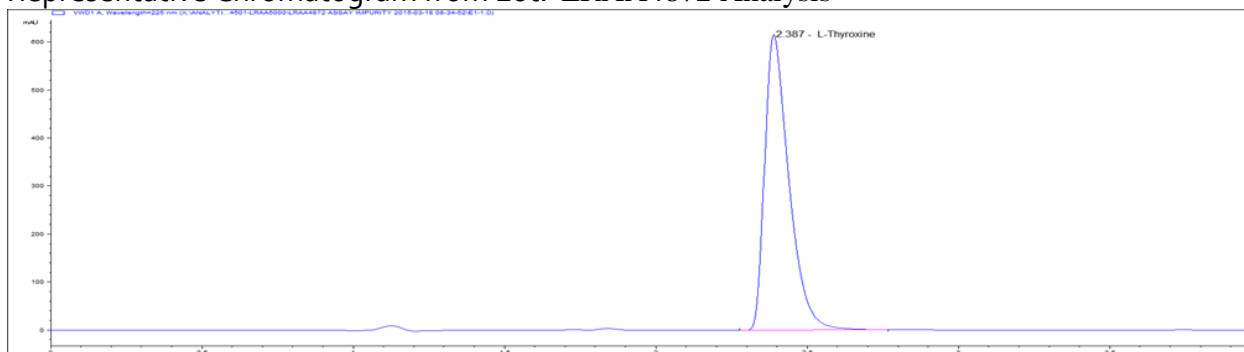
vs. EP BATCH

4.0

Labeled Content = 89.2%, as C₁₅H₁₀L₄NNaO₄

Column: Ascentis Express ES-CN, 4.6 x 100mm, 2.7µm
Mobile Phase: 0.05% H₃PO₄ in Water, 0.05% H₃PO₄ in Acetonitrile (60:40)
Flow Rate: 1mL/min
Column Temperature: 30°C
Injection: 10µL
Detector: 225nm

Representative Chromatogram from Lot: LRAA4872 Analysis



PURITY DETERMINATION BY MASS BALANCE

CHROMATOGRAPHIC IMPURITY ANALYSIS

METHOD: HPLC (ref.: Levothyroxine, EP 7)

Column: Ascentis Express ES-CN, 4.6 x 100mm, 2.7µm

Mobile Phase A: 0.05% H₃PO₄ in Water

Mobile Phase B: 0.05% H₃PO₄ 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: 1mL/min

Column Temperature: 30°C

Injection: 25µL

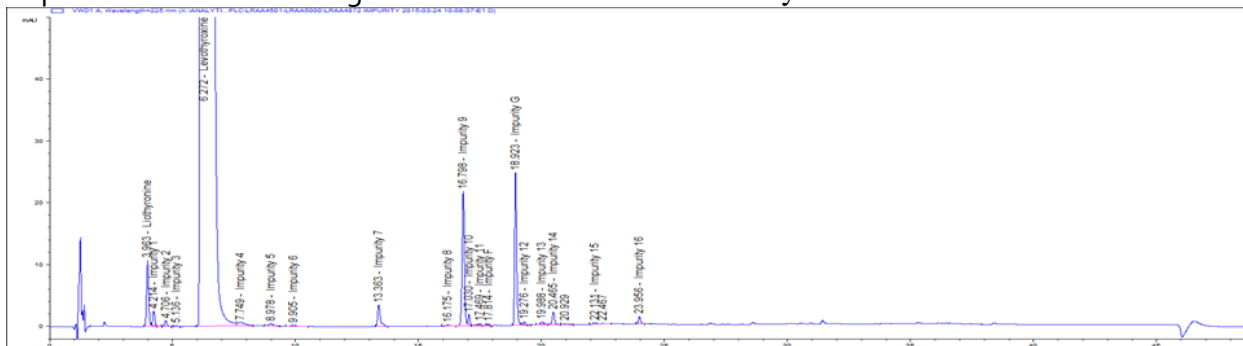
Detector: 225nm

Impurities Detected:

Liothyroxine:	0.3%	Impurity 1:	0.07%
Impurity 2:	0.04%	Impurity 3:	0.01%
Impurity 4:	0.07%	Impurity 5:	0.03%
Impurity 6:	0.01%	Impurity 7:	0.1%
Impurity 8:	0.01%	Impurity 9:	0.8%
Impurity 10:	0.04%	Impurity 11:	0.01%
Impurity F:	0.01%	Impurity G:	0.7%
Impurity 12:	0.01%	Impurity 13:	0.03%
Impurity 14:	0.1%	Impurity 15:	0.02%
Impurity 16:	0.04%		

Total Impurities: **2.5%**

Representative Chromatogram from Lot: LRAA4872 Analysis



RESIDUAL SOLVENTS

Method: GC-MS Headspace (ref.: Adapted from Residual Solvents <467>, USP36)

Column: DB-1301

Carrier gas: He

Flow: 1.2mL/min

Split Ratio: 1:5

Injection/Temperature: 1mL/250°C

Temperature Program: 40°C for 20min, 10°C/min to 240°C, hold 20min

Solvents Detected: None

LOSS ON DRYING/VOLATILES

Method: Under vacuum at 60°C

Mean of three measurements, Loss = **0.4%**

RESIDUE ANALYSIS

Method: Sulfated Ash

Sample Size: ~100mg

Mean of three measurements, Residue = **0.6%**

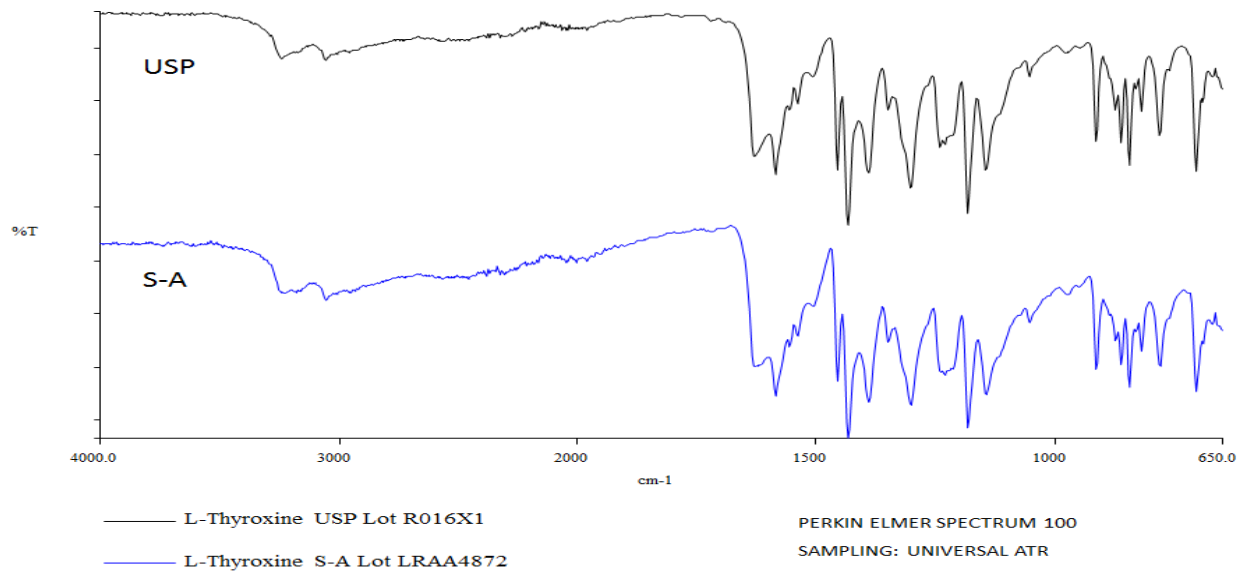
CERTIFIED PURITY BY MASS BALANCE [100% - Impurities (normalized)]

97.0% $U_{\text{crm}} = \pm 0.1\%$, $k = 2$ (dried basis)

96.6% $U_{\text{crm}} = \pm 0.5\%$, $k = 4.30$ (as is basis)

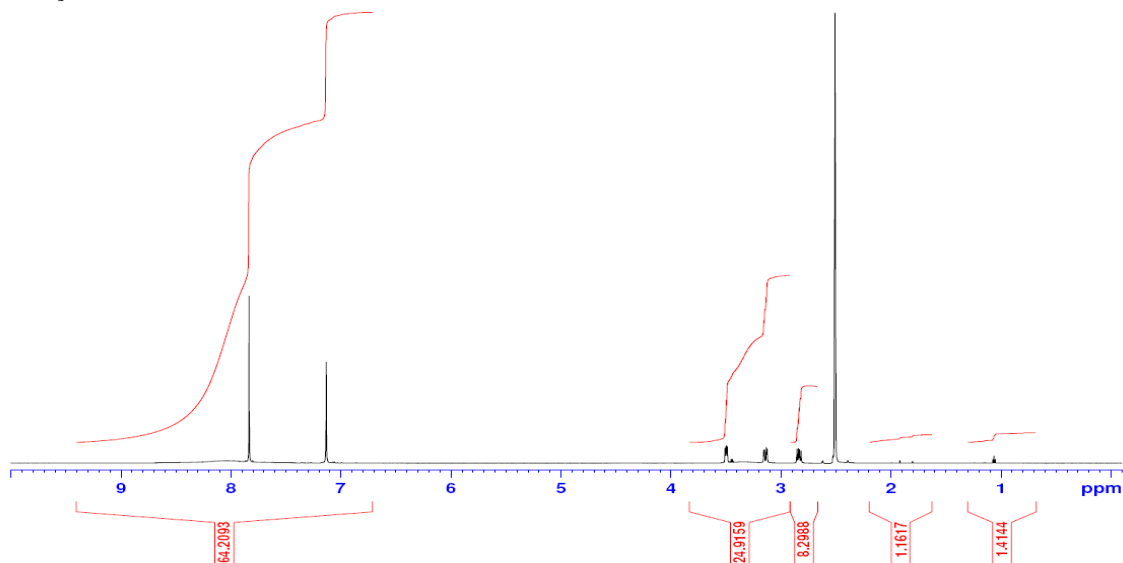
IDENTIFICATION TESTS

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



¹H NMR (Data provided by an external laboratory; not in scope of accreditation)

LRAA4872
L-Thyroxine in DMSO-d₆



Consistent with structure

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: ~25mg

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 Supervisor



QA Supervisor

APPENDIX

Original Release Date: 06 April 2015
Stability Test Date: 31 March 2016
Requalification Test Date: 31 March 2016