Confidential

*A l'attention de l'*Association *Française des* Malades *de la* Thyroïde (AFMT)

Contenu

1. Rapport Sommaire: Développement de Méthode et Résultats d'Analyses

4. Scan de l'emballage des lots testés

5. Certificats d'Analyses

8- Chromatogrammes Obtenus

12- Certificats d'Analyse de Levothyroxine (Certified Reference Material)

Rapport Sommaire

Objet : Développement de méthode analytique pour la séparation des formes énantiomérique D et L de la thyroxine.

Préparation du Standard :

Standard : Levothyroxine SIGMA-ALDRICH lot: LRAA4872 ; pureté : 96.6 %

La solution mère du standard de Lévothyroxine a été d'abord solubilisée dans 10 mM de NaOH dans un mélange MeOH : H_2O (1 :1), puis la dilution suivante de la phase mobile.

Concentration finale : 0.4 mg/mL

Obtention du mélange racémique :

Le mélange racémique a été obtenu selon la procédure suivante :

La racémisation a été réalisée par chauffage à 50 °C pendant 1 h dans de l'acide acétique glacial en présence de 0.2 équivalent molaire de salicylaldéhyde (1).

Le mélange réactionnel a ensuite été évaporé à sec et le résidu redissous dans MeOH - NaOH 10 mM (1: 1 (v / v)).

Condition Chromatographique :

Colonne : CHIRALPAK ZWIX (-) : 3.0 x 250 mm, 3 µm

Température du compartiment de la colonne : 30°C

Mobile phase : Acide Formique à 50 mM plus Diethylamine à 25 mM dans un mélange de :

MeOH-THF-H_2O (45-50-05)

Débit : 0.5 mL/min.

Longueur d'onde de détection: 230 nm.

Volume d'injection : 20 µL

Résultat:

Le premier résultat obtenu été à 100 % Lévothyroxine, mais la solution de l'échantillon a été faite avec seulement un comprimé.

Concentration de la solution de l'échantillon : 0.01 mg/mL en levothyroxine.

Concentration du standard : 0.4 mg/mL.

Recommandation de concentrer plus la solution d'échantillon.

Les tests sur les trois lots reçus par la suite ont été réalisé en solubilisant 14 comprimés dans 10 mL de phase mobile.

- **Lot : 22042120 - Jun 2019- Ancienne Formulation-Levothyrox 100**

Le chromatogramme montre un seul pic celui du lévothyroxine

Nous obtenons : 102.39 µg/comprimé en Lévothyroxine

- **Lot : 22700620- Sept-2018- Nouvelle Formulation- Levothyrox 100**

Le chromatogramme montre deux pics

Nous obtenons : 88.90 µg/comprimé avec le pic du lévothyroxine

Le deuxième pic donne 12.67 µg/comprimé (peut être celui du dextrothyroxine)

- **Lot : 23204220- Avr 2019- Nouvelle Formulation- Levothyrox 100**

Le chromatogramme montre deux pics

Nous obtenons : 72.17 µg/comprimé avec le pic du lévothyroxine

Le deuxième pic donne 18.41 µg/comprimé (peut être celui du dextrothyroxine)

Sommaire des résultats :

Levothyrox 100 Lot #	22042120 Ancienne Formulation	23204220- Nouvelle Formulation	22700620- Nouvelle Formulation
µg lévothyroxine /comprimé	102.39	72	89
Date d'expiration	06-2019	04-2019	09-2018

Hypothèse :

Notre identification du pic du dextrothyroxine dans le standard est basée sur la réaction de racémisation suivi selon l'article cité en référence. Cette identification n'a pas été confirmée par un standard ou un réactif de dextrothyroxine.

Si cette réaction de racémisation n'a pas fonctionné et qu'une autre impureté a été identifiée comme étant la dextrothyroxine dans notre test alors il est possible que le deuxième pic dans les chromatogrammes des lots : 22700620 et 23204220 est celui de la dextrothyroxine.

Référence :

(1) Direct separation and quantitative analysis of thyroxine and triiodothyronine enantiomers in pharmaceuticals by high-performance liquid chromatography
Gika et al. Journal of Chromatography B, 800 (2004) 193–201.

Titulaire de l'AMM :
Merck Santé s.a.s.
37 rue Saint-Romain
69379 Lyon cedex 08

Exploitant :
Merck Serono s.a.s.
37 rue Saint-Romain
69379 Lyon cedex 08

	MATIN	A JEUN
1 cp		
½ cp		

Liste II – Uniquement sur ordonnance

3400934783401

LEVOTHYROX® 100 µg
comprimé sécable
30 comprimés sécables

MERCK

Titulaire de l'AMM :
Merck Santé s.a.s.
37 rue Saint-Romain
69379 Lyon cedex 08

TRIEZ VOS EMBALLAGES

Exploitant :
Merck Serono s.a.s.
37 rue Saint-Romain
69379 Lyon cedex 08

3400930065891

EXP 04/2019
Lot 22204220

Liste II – Uniquement sur ordonnance

MERCK

LEVOTHYROX® 100 µg
comprimé sécable
30 comprimés sécables

Titulaire de l'AMM :
Merck Santé s.a.s.
37 rue Saint-Romain
69379 Lyon cedex 08

TRIEZ VOS EMBALLAGES

Exploitant :
Merck Serono s.a.s.
37 rue Saint-Romain
69379 Lyon cedex 08

3400930065891

EXP 09/2018
Lot 22700820

Liste II – Uniquement sur ordonnance

MERCK

LEVOTHYROX® 100 µg
comprimé sécable
30 comprimés sécables

[redacted]

[redacted]

Certificat d'Analyse / Certificate of Analysis

59AP02A

Réquisition/ Requisition: N/A
Échantillon/ Sample: **Thyroxine**
Lot: 22042120
Code: N/A

Reçu de/Received from:

[redacted]

a été testé pour donner les résultats ci-dessous
was tested to give the results listed below

#	Test	Method	Specifications	Results
1	Dosage Levothyroxine	HPLC	To report	**102 µg/cap**

Remarques/Remarks: Le certificat 59AP02A remplace le certificat 59AP02 émis le 2018.04.30 (correction du nom du client).

[redacted]

Date: 2018.06.06

[redacted]

Date: 2018-06-06

[redacted]

[redacted]

Certificat d'Analyse / Certificate of Analysis

59AP03A

Réquisition/ Requisition: N/A

Échantillon/ Sample: **Thyroxine**

Lot: 22700620

Code: N/A

Reçu de/Received from:

[redacted]

a été testé pour donner les résultats ci-dessous
was tested to give the results listed below

#	Test	Method	Specifications	Results
1	Dosage Levothyroxine	HPLC	To report	89 µg/cap

Remarques/Remarks: Le certificat 59AP03A remplace le certificat 59AP03 émis le 2018.04.30 (correction du nom du client).

[redacted]

Date: 2018.06.06

[redacted]

Date: 2018-06-06

[redacted]

Certificat d'Analyse / Certificate of Analysis

59AP04A

Réquisition/ Requisition: N/A

Échantillon/ Sample: **Thyroxine**

Lot: 23204220

Code: N/A

Reçu de/Received from:

[redacted]

a été testé pour donner les résultats ci-dessous
was tested to give the results listed below

#	Test	Method	Specifications	Results
1	Dosage Levothyroxine	HPLC	To report	72 µg/cap

Remarques/Remarks: Le certificat 59AP04A remplace le certificat 59AP04 émis le 2018.04.30 (correction du nom du client).

[redacted]

Date: 2018.06.06

[redacted]

Date: 2018-06-06

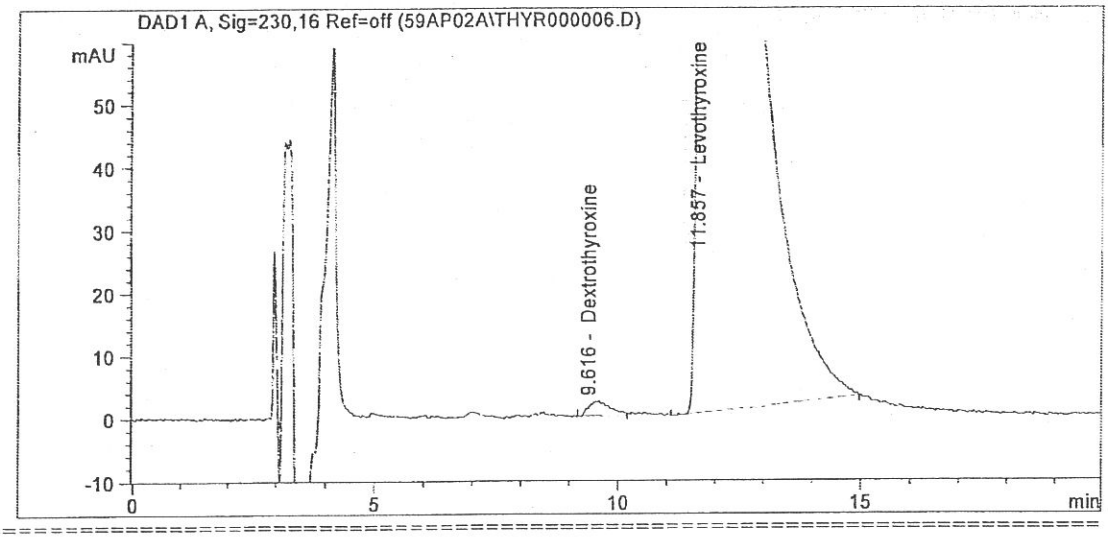
Data file : C:\PROJETS\59AP\59AP02\59AP02A\THYR000006.D
Sample Name: STD A, 5 1

Injection Date	: Mon, 23. Apr. 2018	Seq Line	: 6
Sample Name	: STD A, 5	Location	: Vial 2
Acq Operator	: MB	Inj. No.	: 1
		Inj. Vol.	: 10 µl

Acq. Method : C:\PROJETS\METHODS\THYROXINE.M
Analysis Method : C:\PROJETS\METHODS\THYROXINE.M
Last Changed : Tue, 24. Apr. 2018, 09:59:15 am

Assay Thyroxine enantiomere

->



Customized Report: ASSAY01

Signal Description : DAD1 A, Sig=230,16 Ref=off

#	RT [min]	Type	Area	Height	Area %	Name
1	9.616	BB	74.932	2.491	0.341	Dextrothyroxine
2	11.857	VBA	21925.383	461.402	99.659	Levothyroxine
Totals:						

*** End of Report ***

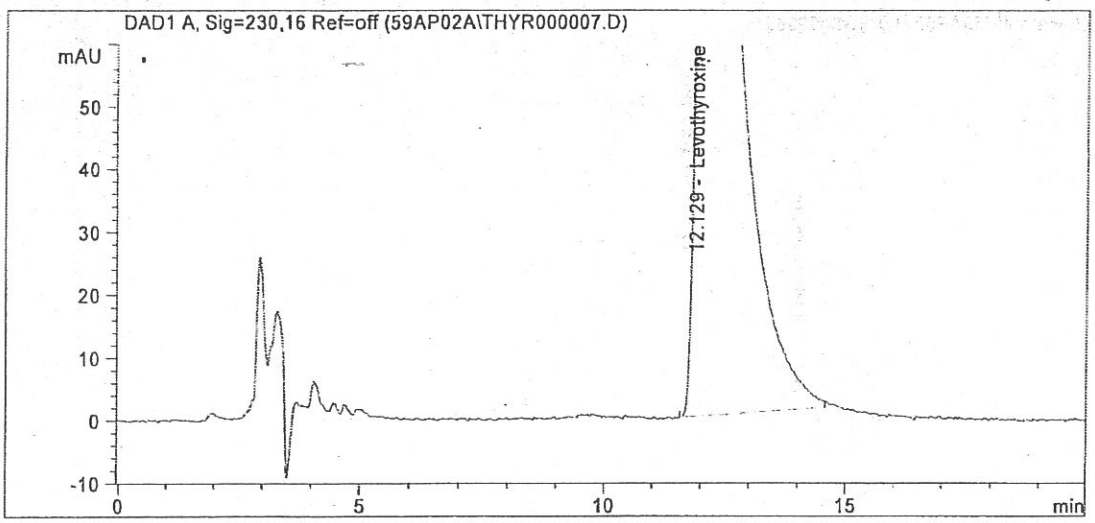
Data file : C:\PROJETS\59AP\59AP02\59AP02A\THYR000007.D
Sample Name: 59AP02 1

Injection Date	: Mon, 23. Apr. 2018	Seq Line	: 7
Sample Name	: 59AP02	Location	: Vial 3
Acq Operator	: MB	Inj. No.	: 1
		Inj. Vol.	: 10 µl

Acq. Method : C:\PROJETS\METHODS\THYROXINE.M
Analysis Method : C:\PROJETS\METHODS\THYROXINE.M
Last Changed : Tue, 24. Apr. 2018, 09:59:15 am

Assay Thyroxine enantiomere

->



Customized Report: ASSAY01

Signal Description : DAD1 A, Sig=230,16 Ref=off

#	RT [min]	Type	Area	Height	Area %	Name
1	0.000		0.000	0.000	0.000	Dextrothyroxine
2	12.129	BB	8368.820	158.280	100.000	Levothyroxine
Totals:						

*** End of Report ***

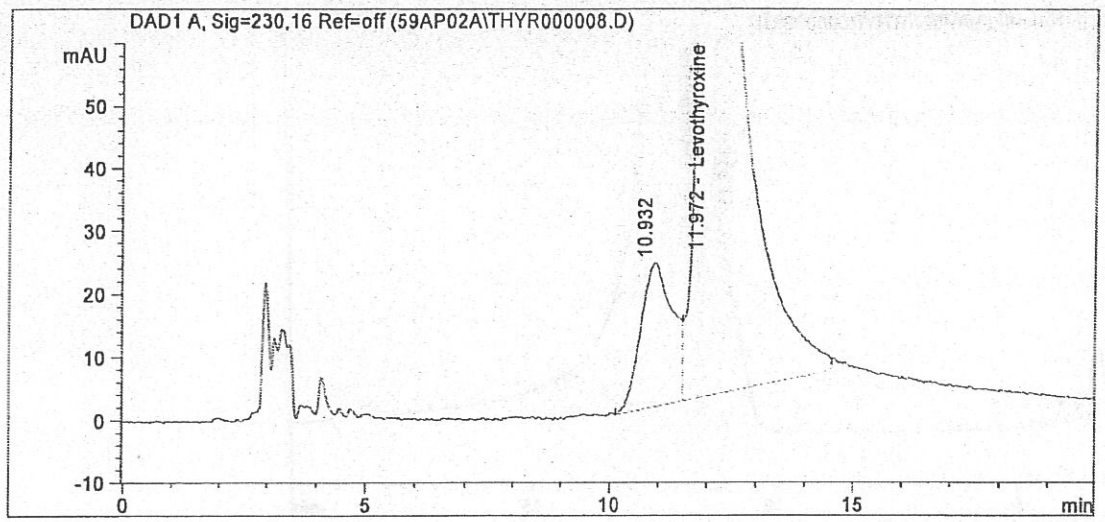
Data file : C:\PROJETS\59AP\59AP02\59AP02A\THYR000008.D
Sample Name: 59AP03 1

Injection Date	: Mon, 23. Apr. 2018	Seq Line :	8
Sample Name	: 59AP03	Location :	Vial 4
Acq Operator	: MB	Inj. No. :	1
		Inj. Vol. :	10 µl

Acq. Method : C:\PROJETS\METHODS\THYROXINE.M
Analysis Method : C:\PROJETS\METHODS\THYROXINE.M
Last Changed : Tue, 24. Apr. 2018, 09:59:15 am

Assay Thyroxine enantiomere

->



Customized Report: ASSAY01

Signal Description : DAD1 A, Sig=230,16 Ref=off

#	RT [min]	Type	Area	Height	Area %	Name
1	0.000		0.000	0.000	0.000	Dextrothyroxine
2	10.932	VV	1036.709	22.797	12.477	
3	11.972	VB	7272.326	122.876	87.523	Levothyroxine

Totals:

*** End of Report ***

Data file : C:\PROJETS\59AP\59AP02\59AP02A\THYR000009.D
Sample Name: 59AP04

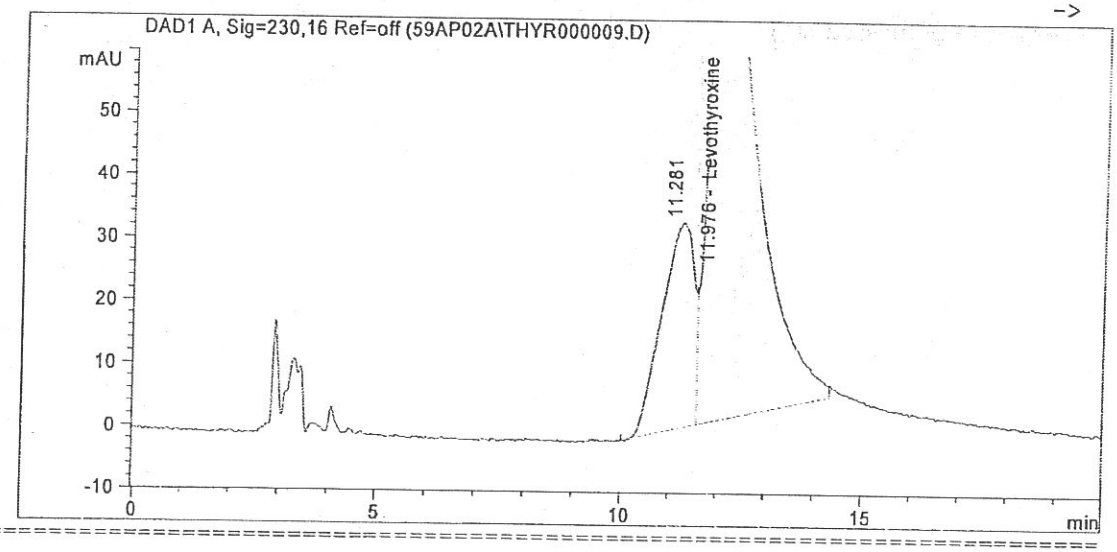
1

Injection Date	: Mon, 23. Apr. 2018	Seq Line	: 9
Sample Name	: 59AP04	Location	: Vial 5
Acq Operator	: MB	Inj. No.	: 1
		Inj. Vol.	: 10 µl

Acq. Method : C:\PROJETS\METHODS\THYROXINE.M
Analysis Method : C:\PROJETS\METHODS\THYROXINE.M
Last Changed : Tue, 24. Apr. 2018, 09:59:15 am

Assay Thyroxine enantiomere

->



Customized Report: ASSAY01

Signal Description : DAD1 A, Sig=230,16 Ref=off

#	RT [min]	Type	Area	Height	Area %	Name
1	0.000		0.000	0.000	0.000	Dextrothyroxine
2	11.281	BV	1506.542	32.583	20.322	
3	11.976	VB	5906.809	95.967	79.678	Levothyroxine

Totals:

*** End of Report ***

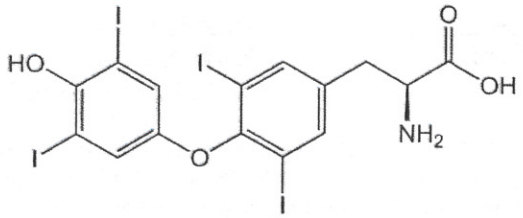
Certificate of Analysis

ISO GUIDE 34
ANAB Cert# AR-1470

ISO/IEC 17025
ANAB Cert# AT-1467

LEVOTHYROXINE

CERTIFIED REFERENCE MATERIAL



CERTIFIED PURITY:

97.0%, U_{crm} = ±0.1% k = 2 (Mass Balance/dried basis)

96.6%, U_{crm} = ±0.5% k = 4.30 (Mass Balance/as is basis)

NOMINAL PACKAGE SIZE: 1g

CATALOG #: PHR1613 **LOT #:** LRAA4872

CERTIFICATE VERSION: LRAA4872.3 **ISSUE DATE:** 19 September 2017

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.

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

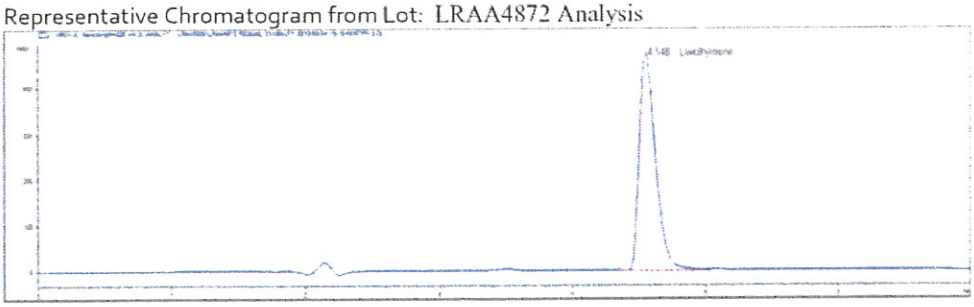
Comparative assay demonstrates direct traceability to Pharmacopeial Standards
Specification: 97.0-103.0% (USP)

ASSAY vs. USP REFERENCE STANDARD (dried basis)

ASSAY VALUE	vs. USP LOT
97.7%	**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% H_3PO_4 in Water, 0.05% H_3PO_4 in Acetonitrile (60:40)
Flow Rate: 0.5mL/min
Column Temperature: 30°C
Injection: 6µL
Detector: 225nm

Representative Chromatogram from Lot: LRAA4872 Analysis

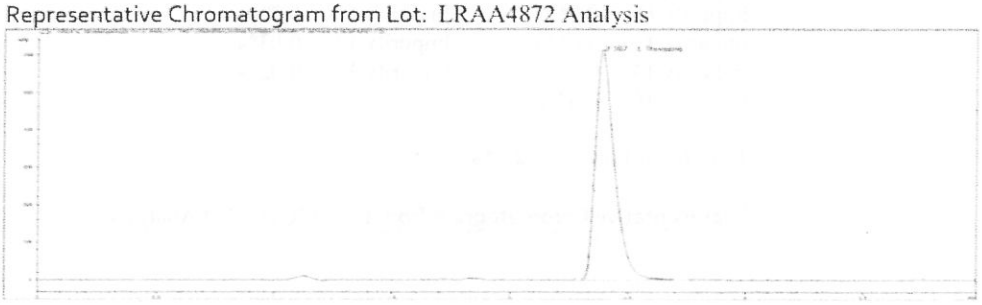


ASSAY vs. EP CRS (as is basis)

ASSAY VALUE	vs. EP BATCH
95.4%	**4.0** Labeled Content = 89.2%, as $C_{15}H_{10}I_4NNaO_4$

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: 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% 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

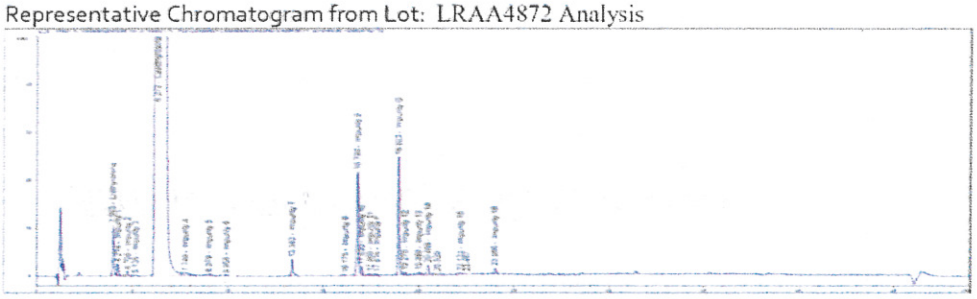
Flow Rate: 1mL/min
Column Temperature: 30°C
Injection: 25µL
Detector: 225nm

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 USP <467>)
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%**

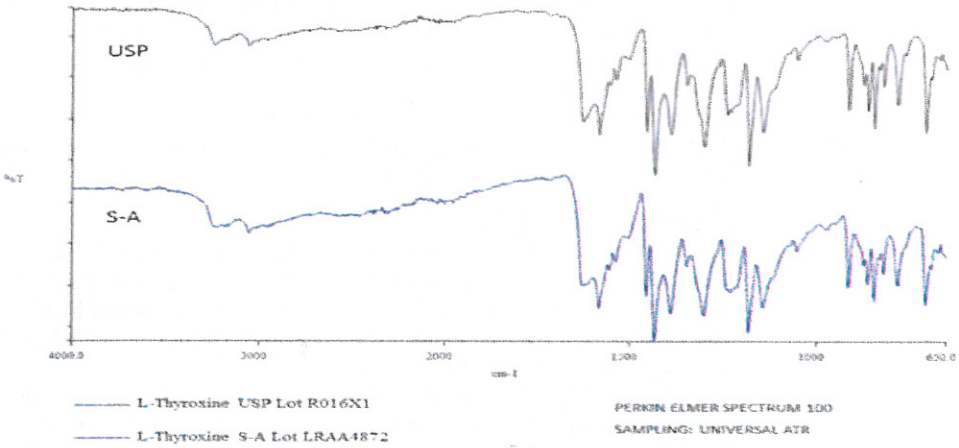
RESIDUE ANALYSIS
Method: Sulfated Ash
Sample Size: ~100mg
Mean of three measurements, Residue = **0.6%**

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

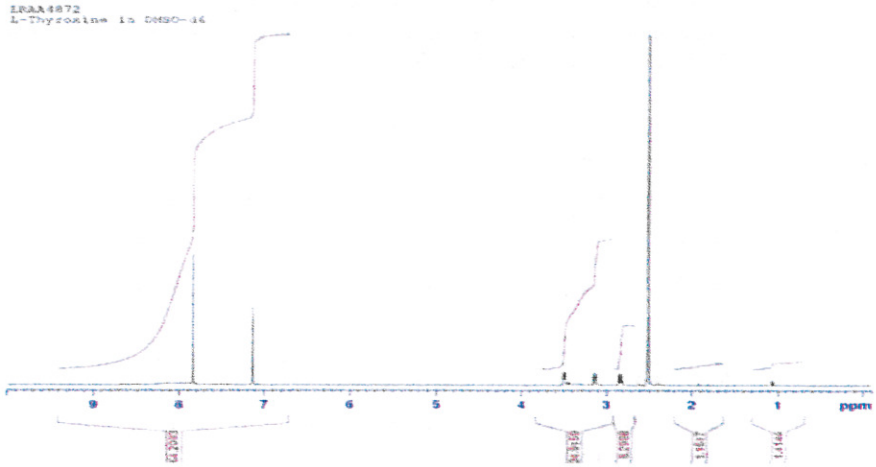
97.0% U_{crm} = ±0.1%, k = 2 (dried basis)
96.6% U_{crm} = ±0.5%, k = 4.30 (as is basis)

IDENTIFICATION TESTS

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



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



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

Manufactured and certified by Sigma-Aldrich RTC, Inc.
2931 Soldier Springs Rd, Laramie WY, USA 82070
(Phone): 1-307-742-5452 (Fax): 1-855-831-9212
email: RTCTechGroup@sial.com