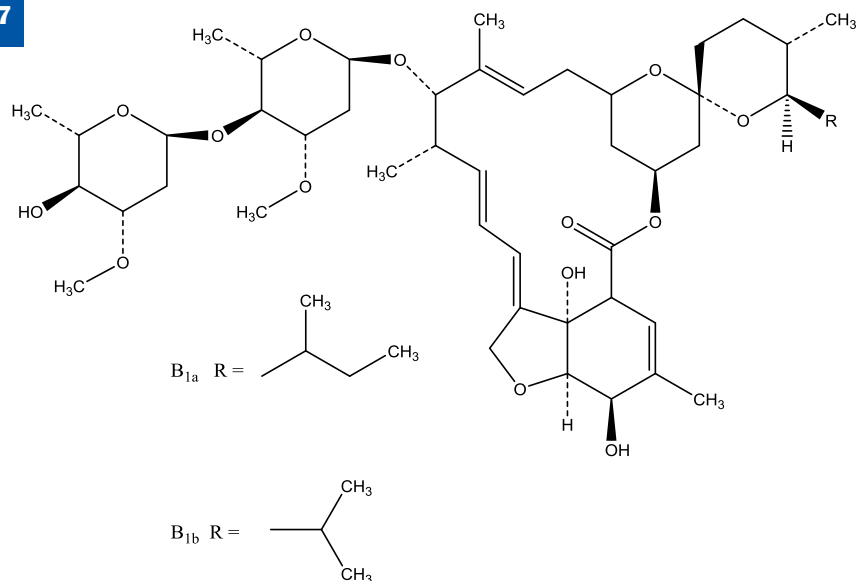


# Certificate of Analysis

ISO 17034  
ANAB Cert# AR-1470

ISO/IEC 17025  
ANAB Cert# AT-1467

## IVERMECTIN CERTIFIED REFERENCE MATERIAL



### CERTIFIED PURITY:

**89.2%**,  $U_{\text{crm}} = \pm 0.3\%$   $k = 2$  (Mass Balance, as  $C_{48}H_{74}O_{14}$ /as is basis)

**2.6%**,  $U_{\text{crm}} = \pm 0.2\%$   $k = 2$  (Mass Balance, as  $C_{47}H_{72}O_{14}$ /as is basis)

NOMINAL PACKAGE SIZE: 1g

CATALOG #: PHR1380

LOT #: LRAB3534

CERTIFICATE VERSION: LRAB3534.3

ISSUE DATE: 30 November 2018

*Note: Certificates may be updated due to Pharmacopeial Lot changes or the availability of new data.*

*Check our website at: [www.sigma-aldrich.com](http://www.sigma-aldrich.com) for the most current version.*

CRM EXPIRATION: 31 December 2021 (Proper Storage and Handling Required).

RECEIPT DATE: \_\_\_\_\_

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

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

**SIGMA-ALDRICH®**

**CHEMICAL FORMULA:** C<sub>48</sub>H<sub>74</sub>O<sub>14</sub> + C<sub>47</sub>H<sub>72</sub>O<sub>14</sub>

**MW:** NA

**PHYSICAL DESCRIPTION:** White powder in amber vial **CAS #:** 70288-86-7

**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:** 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

#### ***ASSAY vs. USP REFERENCE STANDARD (as is basis)***

**ASSAY VALUE**

**vs. USP LOT**

**89.0% (B<sub>1a</sub>)**

**R091C0**

**2.4% (B<sub>1b</sub>)**

Labeled Content = 0.894mg/mg, B<sub>1a</sub>  
0.014mg/mg, B<sub>1b</sub>

#### ***ASSAY vs. EP CRS (as is basis)***

**ASSAY VALUE**

**vs. EP BATCH**

**89.0% (B<sub>1a</sub>)**

**3.0**

**2.4% (B<sub>1b</sub>)**

Labeled Content = None  
Assigned Content = 90.7%, B<sub>1a</sub>  
0.6%, B<sub>1b</sub>\*

\*The assigned content of the EP CRS was determined by assay against the USP Reference Standard

#### **METHOD: HPLC (ref.: Ivermectin, Current Compendial Monographs)**

Column: Ascentis Express C18, 4.6 x 100mm, 5µm

Mobile Phase: Acetonitrile, Methanol, Water (53:27.5:19.5)

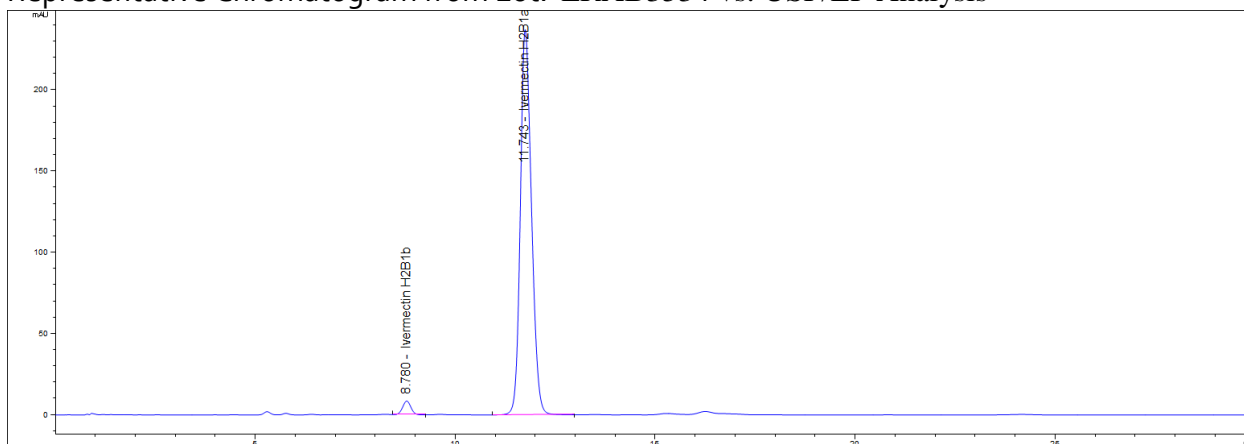
Flow Rate: 1.0 mL/min

Column Temperature: 30 °C

Injection: 10 µL

Detector: 254 nm

## Representative Chromatogram from Lot: LRAB3534 vs. USP/EP Analysis



### ASSAY vs. BP CRS (as is basis)

#### ASSAY VALUE

**89.1% (B<sub>1a</sub>)**

**3.0% (B<sub>1b</sub>)**

#### vs. BP BATCH

**3549**

Labeled Content = 84.7%, B<sub>1a</sub>  
5.9%, B<sub>1b</sub>

### METHOD: HPLC (ref.: Ivermectin, Current Compendial Monographs)

Column: Ascentis Express C18, 4.6 x 100mm, 5µm

Mobile Phase: Acetonitrile, Methanol, Water (53:27.5:19.5)

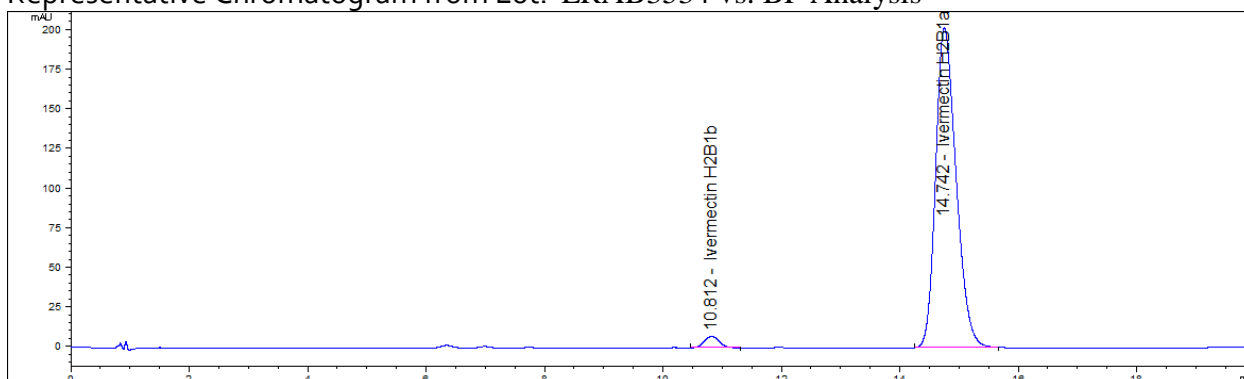
Flow Rate: 1mL/min

Column Temperature: 30°C

Injection: 10µL

Detector: 254nm

## Representative Chromatogram from Lot: LRAB3534 vs. BP Analysis



## **PURITY DETERMINATION BY MASS BALANCE**

### **CHROMATOGRAPHIC IMPURITY ANALYSIS**

**METHOD: HPLC (ref.: Ivermectin, Current Compendial Monographs)**

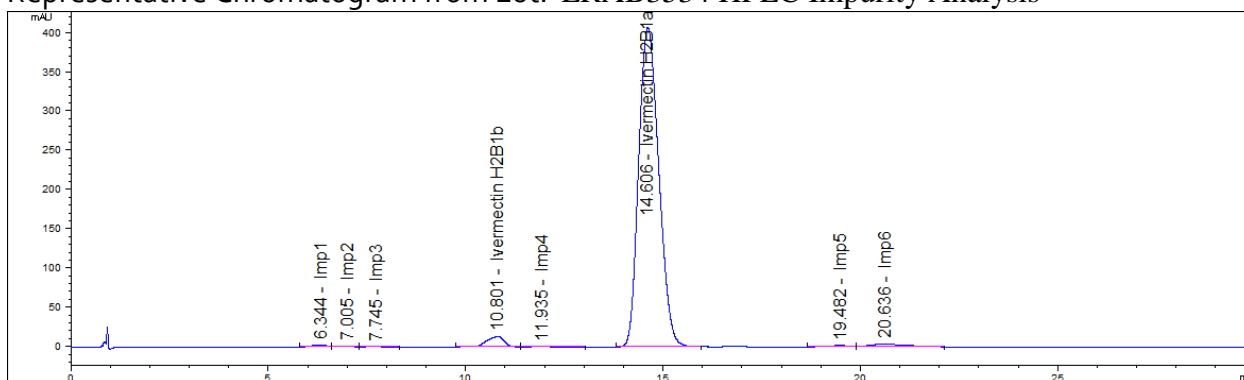
See Assay

Impurities Detected:

Impurity 1:	0.4%
Impurity 2:	0.2%
Impurity 3:	0.1%
Impurity 4:	0.1%
Impurity 5:	0.5%
Impurity 6:	1.6%

Total Impurities: **2.9%**

Representative Chromatogram from Lot: LRAB3534 HPLC Impurity Analysis



**METHOD: GC (ref.: Ivermectin, Current Compendial Monographs)**

Column: SPB-624, 30m x 0.53mm x 3.0µm

Carrier gas: H<sub>2</sub>

Flow: 0.4mL/min

Split Ratio: 10:1

Injection/Temperature: 1µL/220°C

Temperature Program: 40°C for 5min, 20°C/min to 180°C, hold 20min

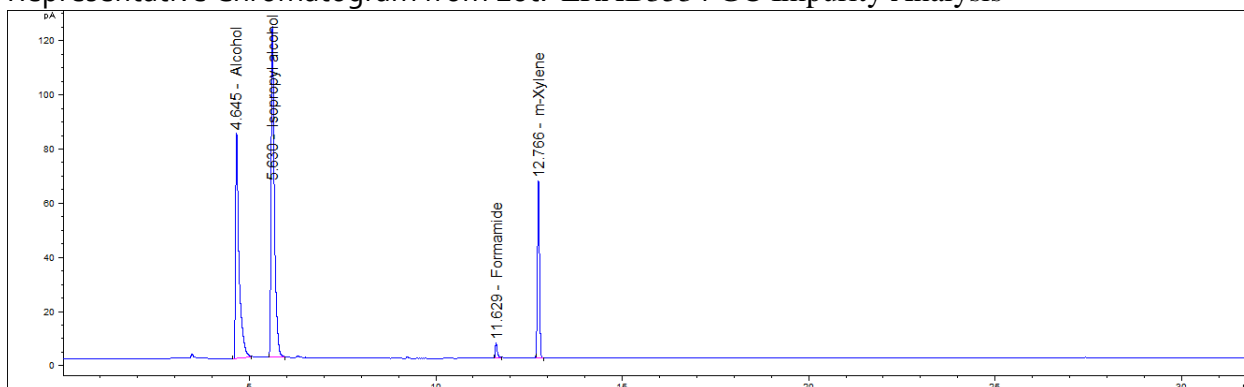
Detector/Temperature: FID/230°C

Internal Standard: Isopropyl Alcohol

Impurities Detected:

Ethanol:	<b>2.9%</b>
Formamide:	<b>2.3%</b>

## Representative Chromatogram from Lot: LRAB3534 GC Impurity 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

### WATER DETERMINATION

Method: Karl Fisher titration (ref.: Current Compendial Monographs)

Mean of three measurements, Water Content = **0.05%**

### RESIDUE ANALYSIS

Method: Sulfated Ash (ref.: Current Compendial Monographs)

Sample Size: ~1g

Mean of three measurements, Residue = **0.02%**

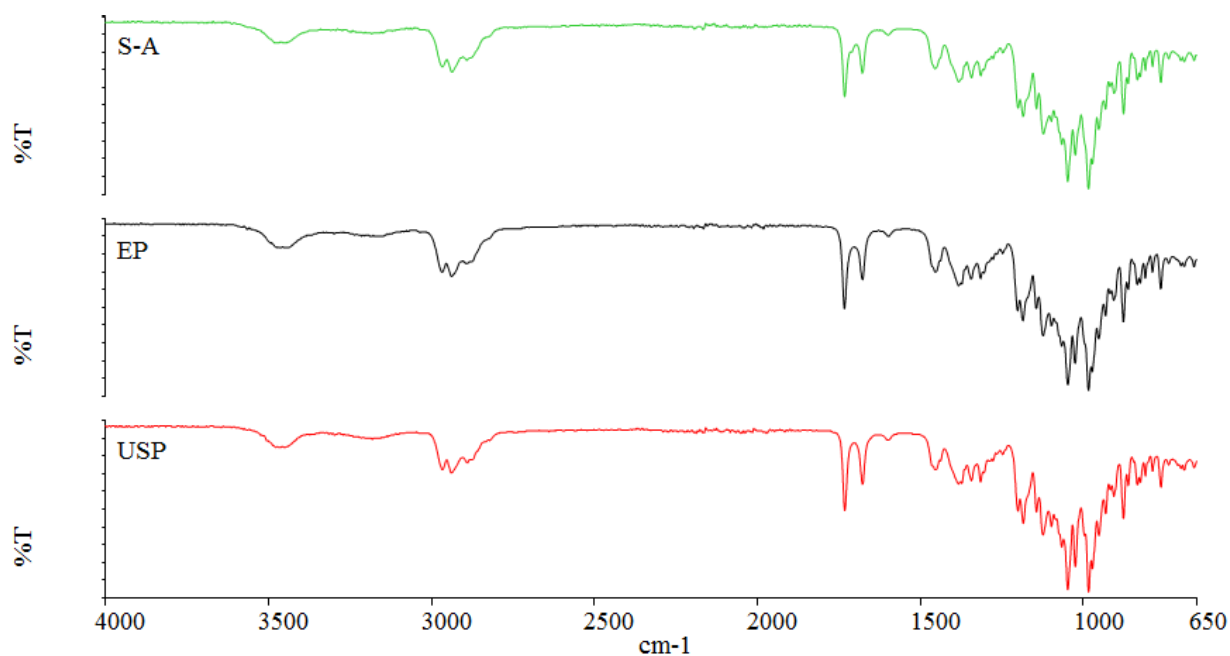
### CERTIFIED PURITY BY MASS BALANCE [100% - Impurities (normalized)]

**89.2%**  $U_{\text{crm}} = \pm 0.3\%$ ,  $k = 2$  (as  $\text{C}_{48}\text{H}_{74}\text{O}_{14}$ , as is basis)

**2.6%**  $U_{\text{crm}} = \pm 0.2\%$ ,  $k = 2$  (as  $\text{C}_{47}\text{H}_{72}\text{O}_{14}$ , as is basis)

## IDENTIFICATION TESTS

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



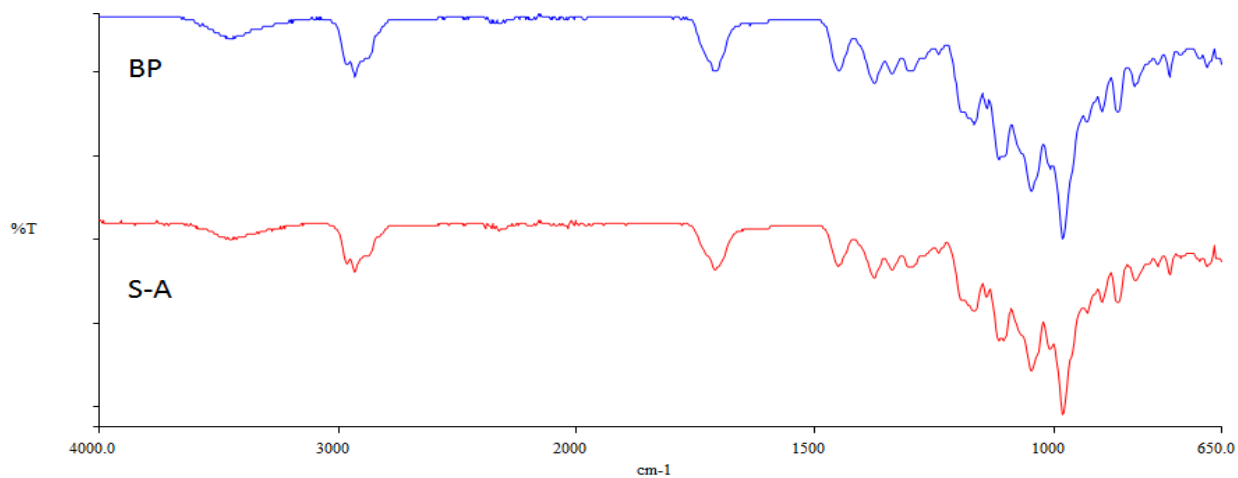
— Ivermectin S-A Lot LRAB3534

— Ivermectin EP Batch 3.0

— Ivermectin USP Lot R091C0

PERKIN ELMER SPECTRUM 100

SAMPLING: UNIVERSAL ATR



— Ivermectin BP Batch 3549

— Ivermectin S-A Lot LRAB3534

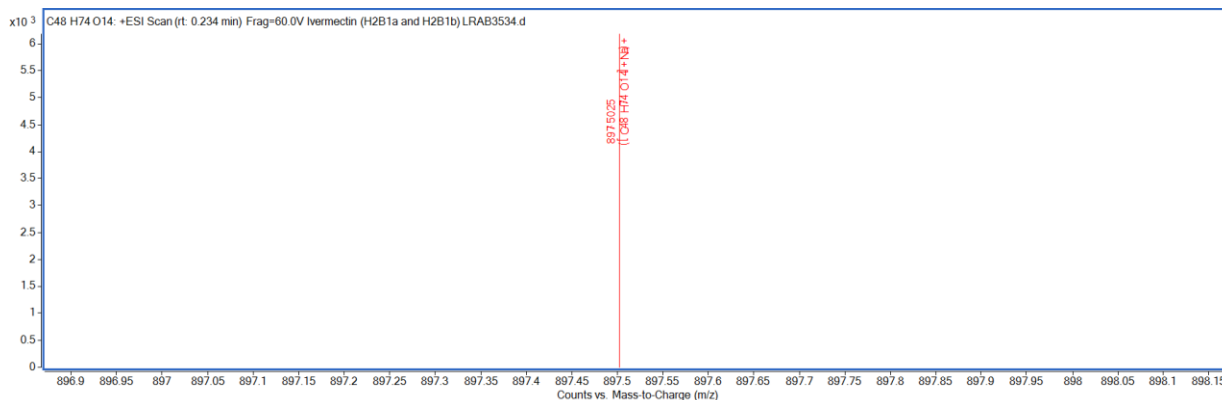
PERKIN ELMER SPECTRUM 100

SAMPLING: UNIVERSAL ATR

Recrystallized in MeOH

## MASS SPECTRUM

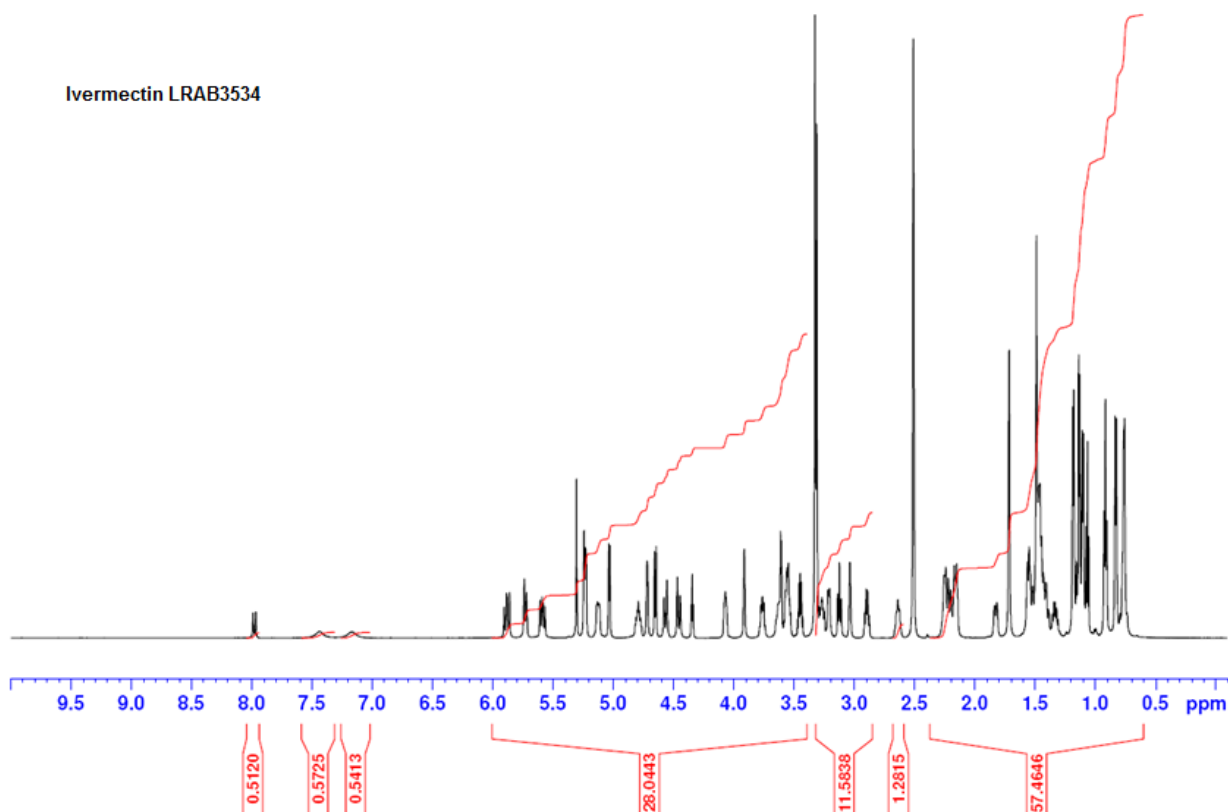
Method: HR-QTOF; 4.0 kV ESI+; temperature: 325 °C



Theoretical value: 897.4976  $m/z$

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

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



Consistent with structure

## ELEMENTAL ANALYSIS (Data provided by an external laboratory; not in scope of accreditation)

Exeter Analytical 440 Elemental Analyzer

Combustion method

%	Theoretical	Result 1	Result 2	Mean
C	65.88	64.68	64.66	64.67
H	8.52	8.46	8.50	8.48

## OPTICAL ROTATION

Specification:  $-17^{\circ}$  to  $-20^{\circ}$  (USP)

Perkin Elmer Polarimeter 343

Wavelength: 589nm

Concentration: 2.3g/100mL

Cell Path: 100mm

Mean of three Measurements =  $-18^{\circ}$

## 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: ~20mg

## UNCERTAINTY STATEMENT


Uncertainty values in this document are expressed as Expanded Uncertainty ( $U_{\text{crm}}$ ) corresponding to the 95% confidence interval.  $U_{\text{crm}}$  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.



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QC Manager



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Head Quality Assurance

#### APPENDIX

Original Release Date:	22 November 2016
Requalification Test Date:	31 December 2016
Stability Test Date:	30 November 2018
Requalification Test Date:	30 November 2018