

Certificate of Analysis

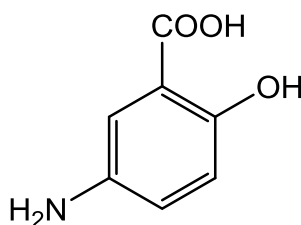
ISO GUIDE 34

ANAB Cert# AR-1470

ISO/IEC 17025

ANAB Cert# AT-1467

MESALAMINE (Mesalazine) CERTIFIED REFERENCE MATERIAL



CERTIFIED PURITY:

99.9%, $U_{\text{crm}} = \pm 0.1\%$ $k = 2$ (Mass Balance/dried basis)

99.8%, $U_{\text{crm}} = \pm 0.1\%$ $k = 2$ (Mass Balance/as is basis)

NOMINAL PACKAGE SIZE: 1g

CATALOG #: PHR1060

LOT #: LRAA7173

CERTIFICATE VERSION: LRAA7173.3

ISSUE DATE: 28 February 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 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: C₇H₇NO₃

MW: 153.1

PHYSICAL DESCRIPTION: White powder in amber vial **CAS #:** 89-57-6

SIGMA-ALDRICH®

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 Laboratory Use only. Not for drug, household or other uses.

TRACEABILITY ASSAY

Comparative assay demonstrates direct traceability to Pharmacopeial Standards

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

ASSAY vs. USP REFERENCE STANDARD (as is basis)

ASSAY VALUE

99.9%

vs. USP LOT

R001T0

Labeled Content = 0.999 mg/mg

Column: Ascentis Express C18, 4.6 x 100mm, 5 μ m

Mobile Phase: Buffer, methanol; (81:19)

Buffer: 7.1g dibasic sodium phosphate + 6.9 g monobasic sodium phosphate + 7.5mL tetrabutylammonium hydroxide in methanol (1 in 4), dilute with water to 1000 volume.

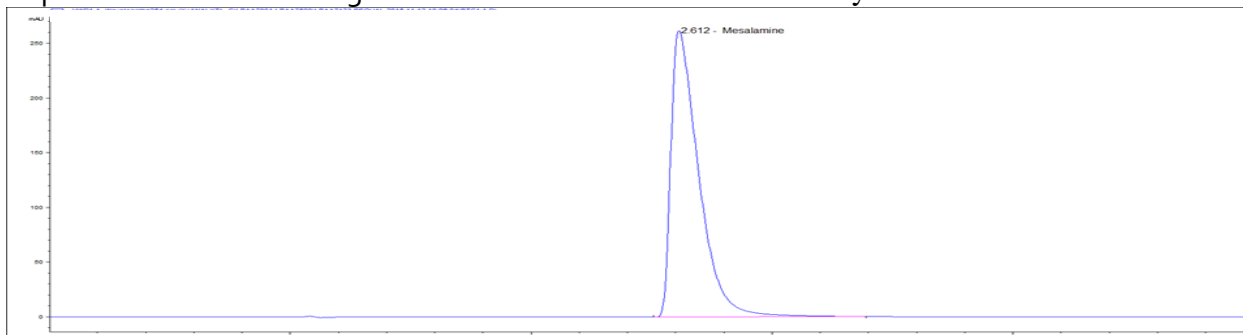
Flow Rate: 0.8mL/min

Column Temperature: 30°C

Injection: 2 μ L

Detector: 254 nm

Representative Chromatogram from Lot: LRAA7173 USP Analysis



ASSAY vs. EP CRS (as is basis)

ASSAY VALUE

99.9%

vs. EP BATCH

3.0

Labeled Content = None

Assigned Content = 100.1%*

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

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

Mobile Phase: Buffer, methanol; (85:15)

Buffer: See USP Assay

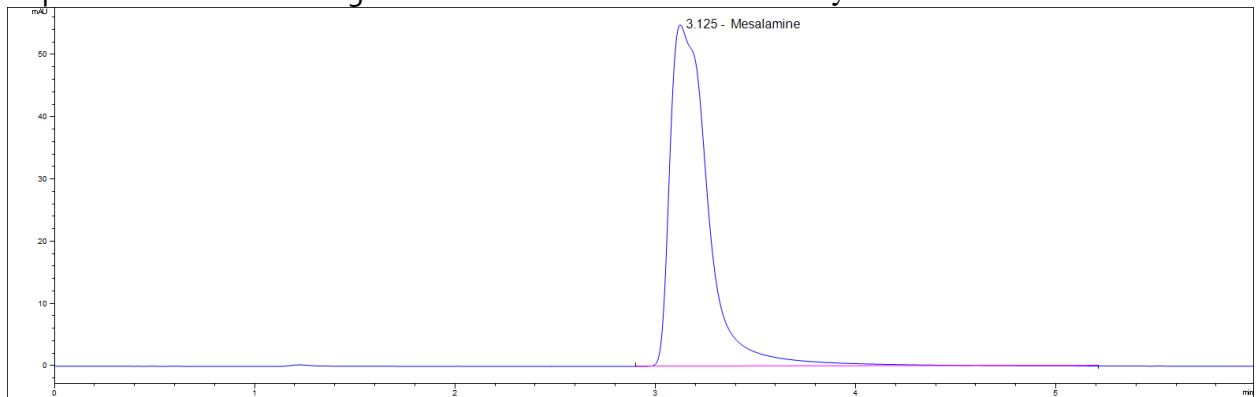
Flow Rate: 0.8 mL/min

Column Temperature: 30°C

Injection: 2 µL

Detector: 254 nm

Representative Chromatogram from Lot: LRAA7173 EP Analysis



METHOD: HPLC

ASSAY vs. BP CRS (as is basis)

ASSAY VALUE

100.0%

vs. BP Batch

3391

Labeled Content = 99.2%

Column: Supelcosil LC-18, 4.6 x 250mm, 5µm

Mobile Phase: Buffer, methanol; (85:15) Buffer: dibasic sodium phosphate, monobasic sodium phosphate, tetrabutylammonium hydroxide in methanol, water

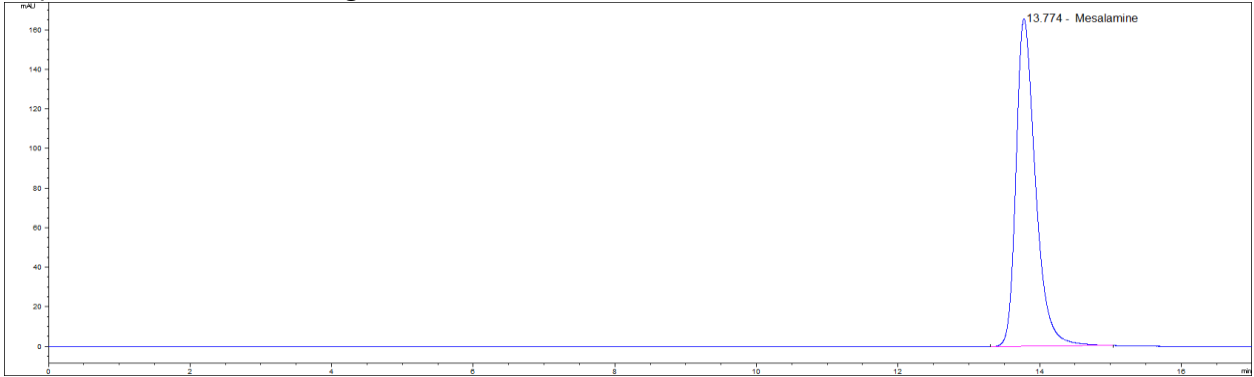
Flow Rate: 1.5mL/min

Column Temperature: 30°C

Injection: 15µl

Detector: 254nm

Representative Chromatogram from Lot: LRAA7173 BP Analysis



PURITY DETERMINATION BY MASS BALANCE

CHROMATOGRAPHIC IMPURITY ANALYSIS

METHOD 1: HPLC (ref.: Mesalamine, Current Compendial Monographs)

Column: Wakosil II C8, 4.6 x 250mm, 5 μ m

Mobile Phase: Buffer, methanol, acetonitrile; (89:8:3) Buffer: monobasic potassium phosphate, sodium 1-octanesulfonate, water, pH 2.2 with phosphoric acid

Flow Rate: 1mL/min

Column Temperature: 27°C

Injection: 20 μ l

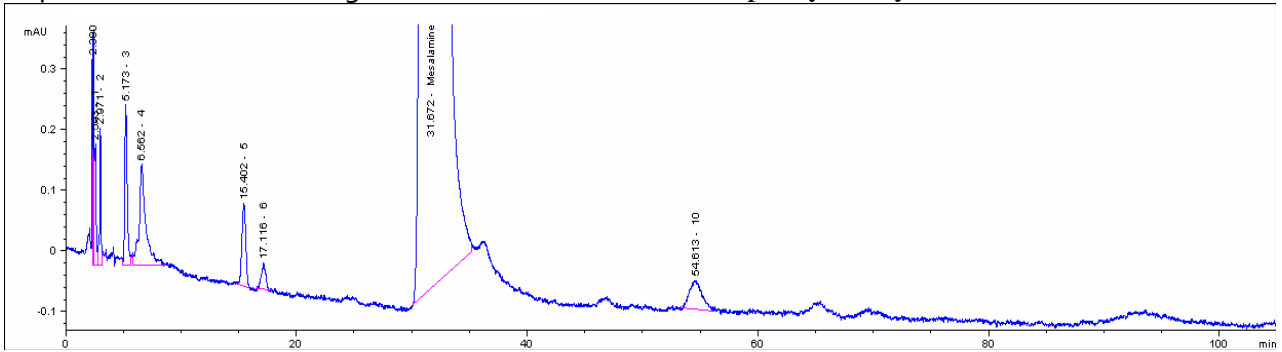
Detector: 220nm

Impurities Detected:

Impurity 1:	0.006%	Impurity 2:	0.006%
Impurity 3:	0.01%	Impurity 4:	0.02%
Impurity 5:	0.01%	Impurity 6:	0.005%
Impurity 7:	0.02%	Impurity 8:	0.01%
2-Aminosalicylic acid:	0.005%		

Total Impurities: **0.09%**

Representative Chromatogram from Lot: LRAA7173 Impurity Analysis



METHOD 2: GC (ref.: Mesalamine, Current Compendial Monographs)

Column: AT-5MS, 0.25mm x 30m x 0.25 μ m

Carrier: He

Flow: 2mL/min

Split Ratio: 1:50

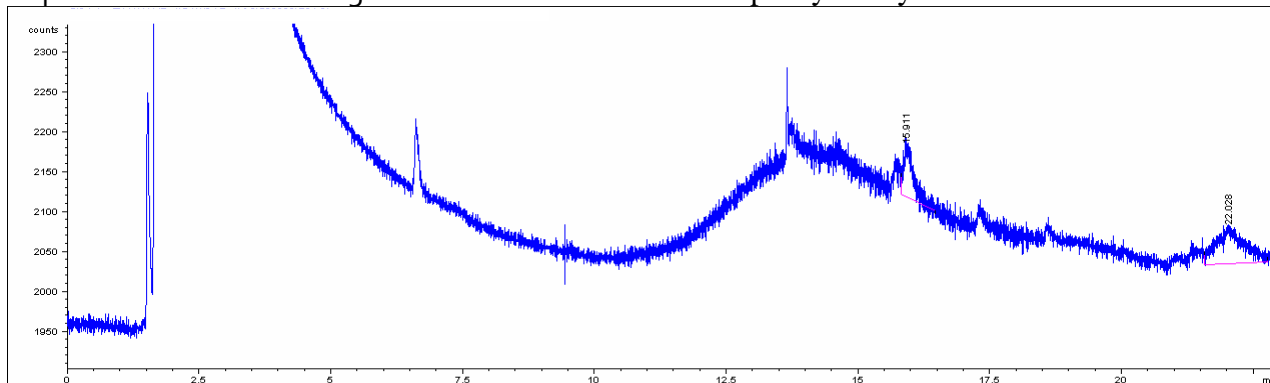
Temperature Program: 40°C/10min, 20°C/min to 200°C, hold 5min

Injection: 2 μ /280°C

Detector: FID/300°C

Impurities Detected: **None Detected**

Representative Chromatogram from Lot: LRAA7173 Impurity Analysis



RESIDUAL SOLVENTS

Method: GC-MS Headspace (ref.: Residual Solvents USP <467>)

Column: DB-1301

Carrier gas: He

Flow: 1.2mL/min

Split Ratio: 1:5

Injection/Temperature: 1 μ L/250°C

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

Solvents Detected: None

LOSS ON DRYING/VOLATILES

Method: Oven at 105°C

Mean of three measurements, Loss = **0.1%**

RESIDUE ANALYSIS

Method: Sulfated Ash

Sample Size: ~1g

Mean of three measurements, Residue = **0.03%**

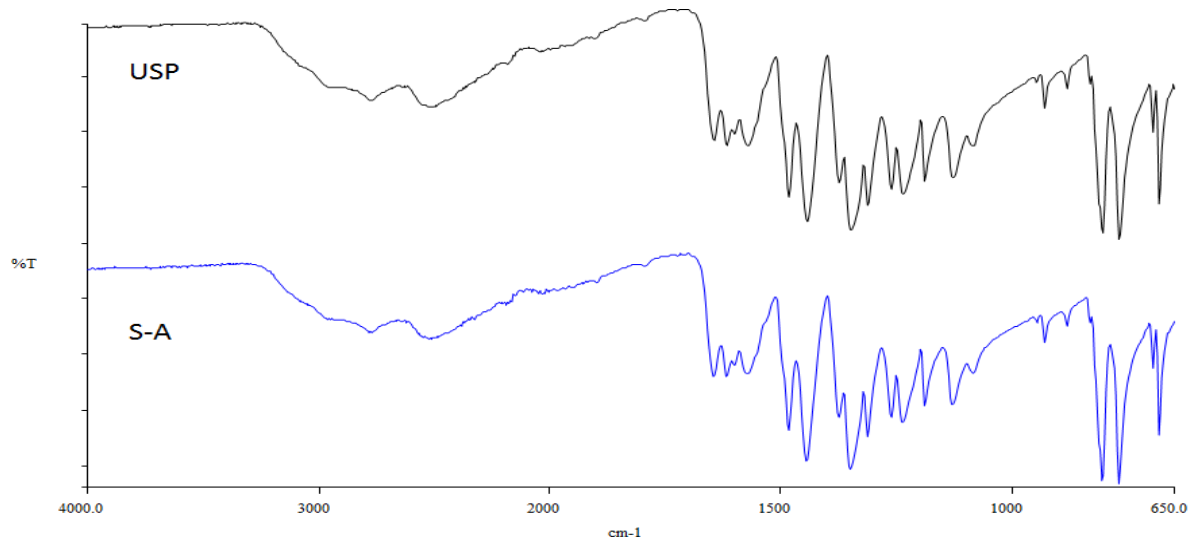
CERTIFIED PURITY BY MASS BALANCE [100% - Impurities (normalized)]

99.9% $U_{\text{crm}} = \pm 0.1\%$, $k = 2$ (dried basis)

99.8% $U_{\text{crm}} = \pm 0.1\%$, $k = 2$ (as is basis)

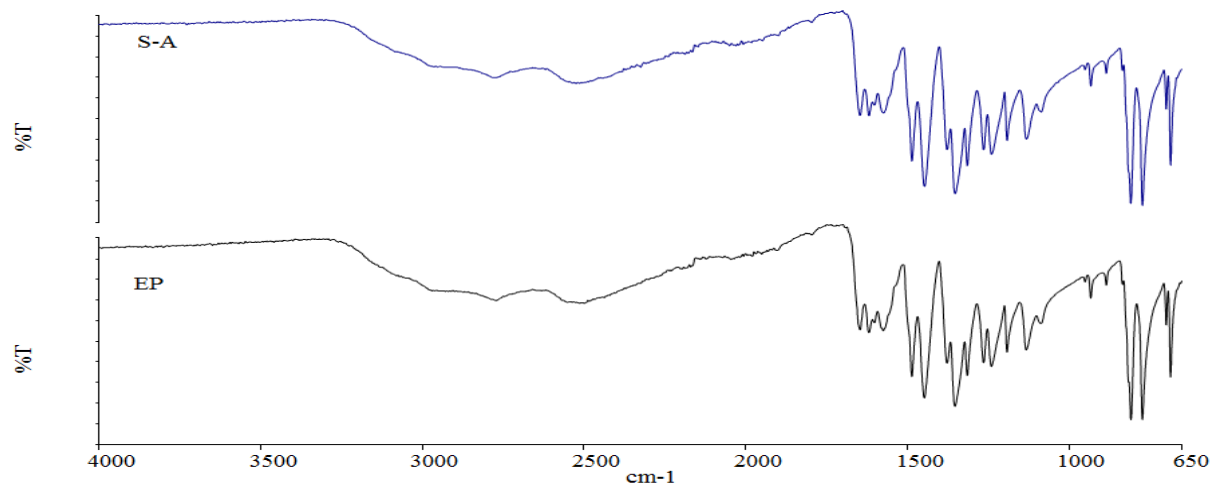
IDENTIFICATION TESTS

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



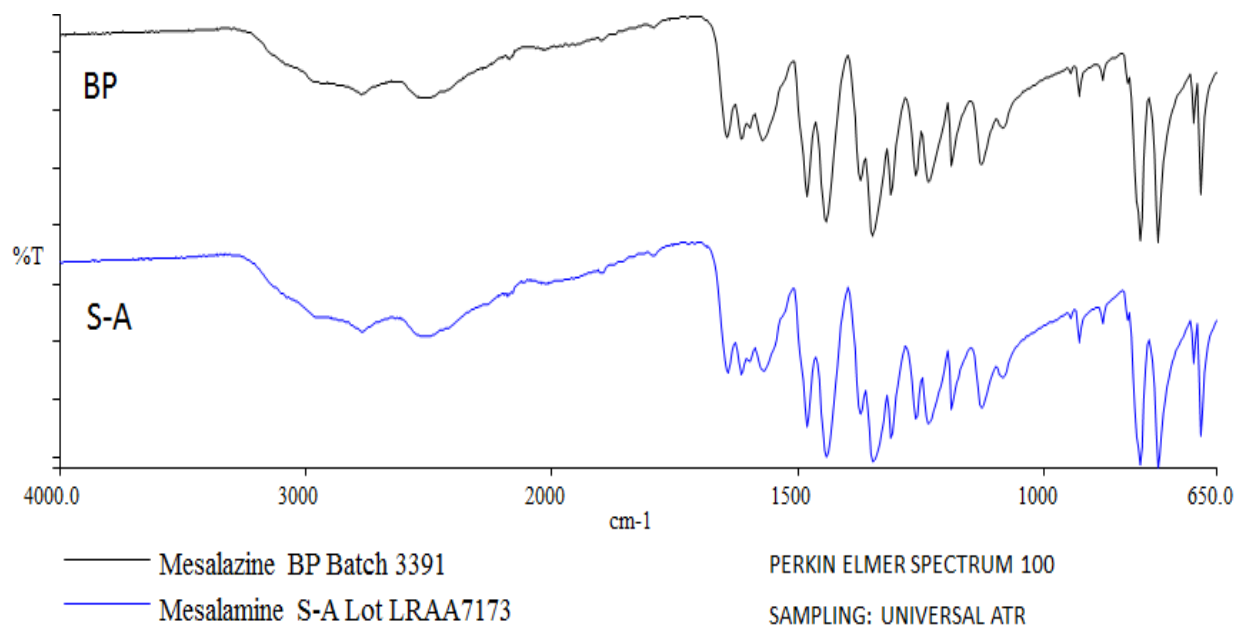
— Mesalamine USP Lot R001T0
— Mesalamine S-A Lot LRAA7173

PERKIN ELMER SPECTRUM 100
SAMPLING: UNIVERSAL ATR



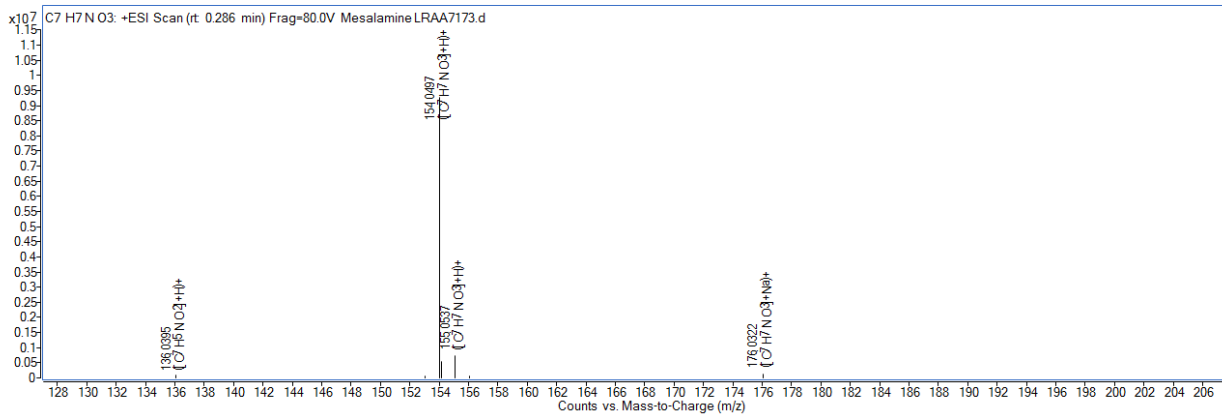
— Mesalamine S-A Lot LRAA7173
— Mesalamine EP Batch 3.0

PERKIN ELMER SPECTRUM 100
SAMPLING: UNIVERSAL ATR



MASS SPECTRUM

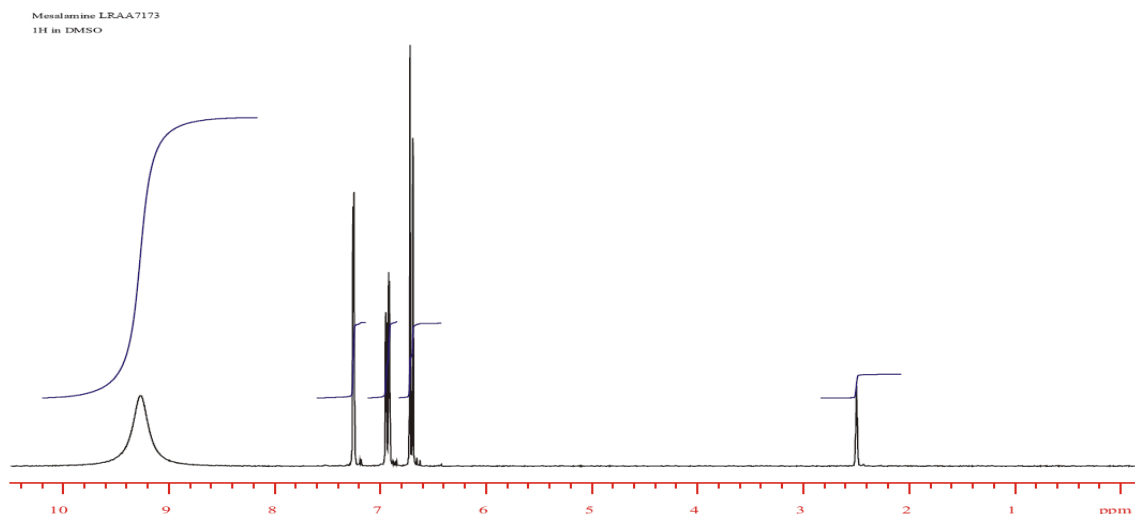
Method: HR-QTOF; 4.0 kV ESI+; temperature: 325 °C



Theoretical value: 154.0504 m/z

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

¹H 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	54.90	54.79	54.85	54.82
H	4.61	4.65	4.57	4.61
N	9.15	9.08	8.99	9.04

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: ~ 40 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



QA Supervisor

APPENDIX

Original Release Date:	19 January 2015
Requalification Test Date:	30 November 2015
Stability Test Date:	28 February 2018
Requalification Test Date:	28 February 2018