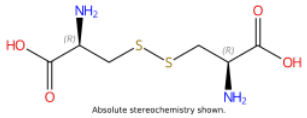
Certificate of Analysis - Certified Reference Material

L-Cystine



Product no.: PHR1323-500MG
Lot no.: **LRAD8501**
Description of CRM: White powder
Expiry date: August 2028
Storage: ROOM TEMPERATURE (2 °C to 30 °C)
Certificate version: LRAD8501.01 (*Note: Certificates may be updated due to the availability of new data. Check our website at: www.sigma-aldrich.com for the most current version.)*
Chemical formula: C6H12N2O4S2
Molecular mass: 240.3
CAS No. 56-89-3

Analyte	Purity (qNMR / as is basis)
L-Cystine	**99.2 % U_{CRM} = ± 0.4 , k = 2.0 (qNMR / as is basis)**

Metrological traceability: Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. When applicable, additional traceability to Primary Standards is established through comparative assay determinations. See "Details on metrological traceability" on page 2.

Measurement method: Where applicable, the certified value is based on a purity determination by mass balance. See section "Certification process details".

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

Minimum sample size: 15 mg

Health and safety information: All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel. Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.

Accreditation: Sigma-Aldrich RTC is accredited by the US accreditation authority ANAB as a registered reference material producer AR-1470 in accordance with ISO 17034.

Certificate issue date: 22-Aug-2024

ANAB ACCREDITED ISO 17034 REFERENCE MATERIAL PRODUCER

[Andy Ommen - QC Authority]

[Christopher Rucinski - QA Authority]

Sigma-Aldrich RTC, 2931 Soldier Springs Rd. Laramie, WY 82070, USA;
Tel. 1 307-742-5452; Fax 1 307-855-831-9211; www.sigmaaldrich.com
Sigma-Aldrich RTC is a subsidiary of Merck KGaA, Darmstadt, Germany.

Instructions for handling and correct use: 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. All values reported on the CoA are for the contents of the unopened standard and apply to the initial use of the standard. Any unused portions remaining after the container has been opened should be carefully stored in accordance with prudent laboratory procedures. Many variables are outside of the control of MilliporeSigma. Therefore, MilliporeSigma makes no warranties concerning the continued suitability of previously opened CRMs. Decisions concerning the proper use of previously opened CRMs are the responsibility of the user.
Expiration is at end of month given on certificate and label.

Packaging: vial of 500 mg

Details on metrological traceability: This standard has been gravimetrically prepared using balances that have been fully qualified and calibrated to ISO 17025 requirements. All calibrations utilize NIST traceable weights which are calibrated externally by a qualified ISO 17025 accredited calibration laboratory to NIST standards. Qualification of each balance includes the assignment of a minimum weighing by a qualified and ISO 17025 accredited calibration vendor taking into consideration the balance and installed environmental conditions to ensure compliance with USP tolerances of NMT 0.10% relative error. Fill volume to predetermined specifications is gravimetrically verified throughout the dispensing process using qualified and calibrated balances. Further traceability to a corresponding Primary Standard may be achieved through a direct comparison assay. Where a Primary Standard is available, the assay value will be included in the specified section of the COA.

Associated uncertainty: 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. If k is not provided, assume a value of 2.0. 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.

$$U_{crm} = \left(\sqrt{u^2_{characterization} + u^2_{homogeneity} + u^2_{stability}}\right) \times k$$

TRACEABILITY ASSAY

Comparative assay demonstrates direct traceability to Pharmacopeial Standards

ASSAY vs. USP REFERENCE STANDARD* 1161586 *(as is basis)

ASSAY VALUE	**vs. USP LOT**
99.8 %	**R19610** Labeled Content = 1.00 mg/mg

ASSAY vs. EP CRS* C3300000 *(as is basis)

ASSAY VALUE	**vs. EP BATCH**
99.8 %	**3.0** Labeled Content = none Assigned Content = 99.5 %*

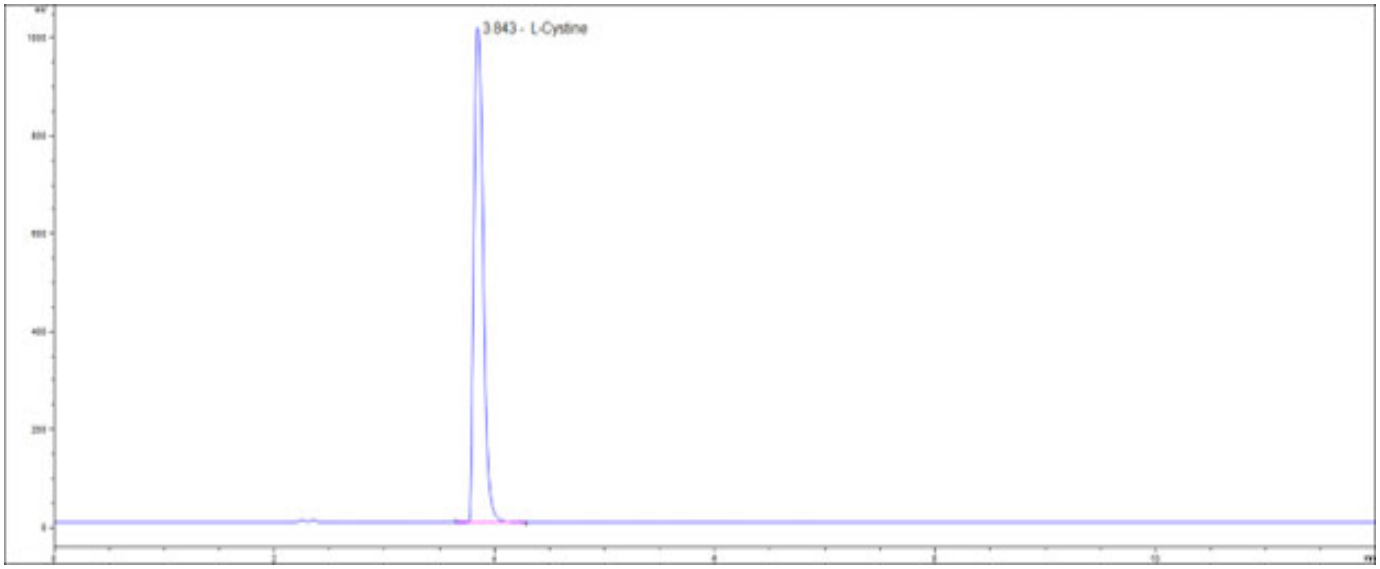
*The assigned content of the EP CRS was determined by assay against the USP Reference Standard

METHOD: HPLC (In House 1772)

Column: Ascentis Express OH5, 150 mm x 4.6mm, 2.7µm particle size
Mobile Phase A: 5mM Ammonium Formate in Water (pH 3.15)
Mobile Phase B: Acetonitrile

Mobile Phase Ratio: 50: 50 (A: B)
Flow Rate: 0.6 mL/min
Column Temperature: 35 °C
Injection Volume: 6 µL
Detector: ELSD

Representative Chromatogram from MilliporeSigma Lot:LRAD8501 Analysis



ADDITIONAL ASSAYS

Titration Assay
Method: Back titration with 0.1N Sodium Thiosulfate

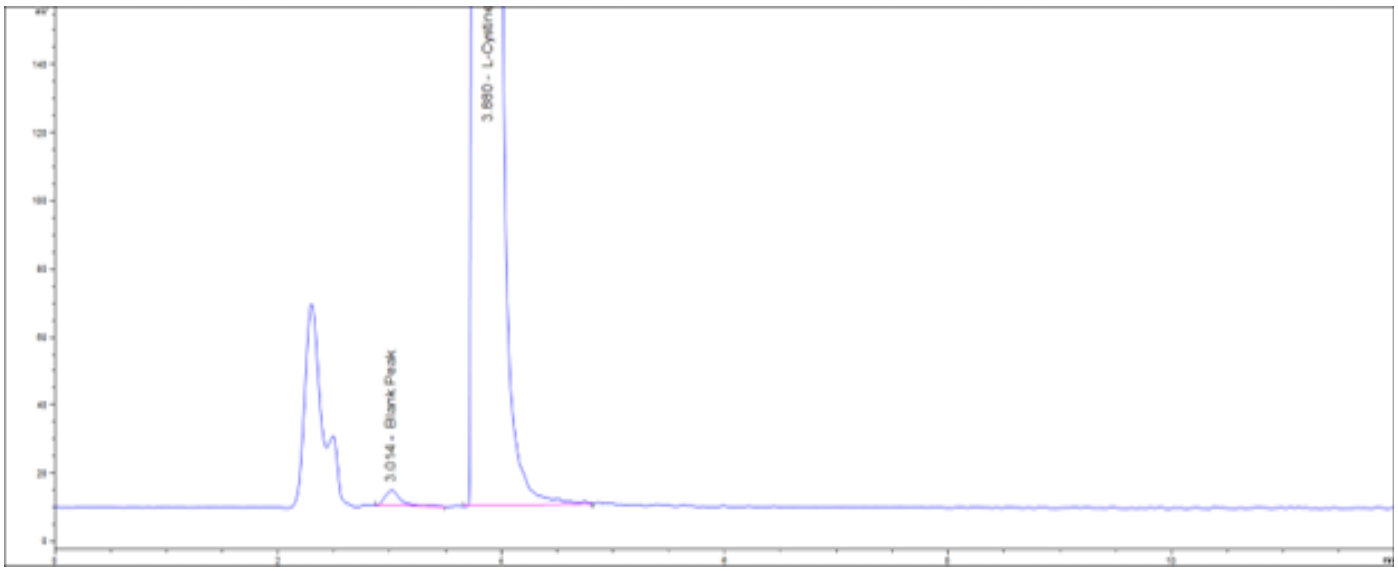
Mean of measurements: **99.1 %**

CHROMATOGRAPHIC IMPURITY ANALYSIS

METHOD: HPLC (In House 1772)

Column: Ascentis Express OH5, 150 mm x 4.6mm, 2.7µm particle size
Mobile Phase A: 5mM Ammonium Formate in Water (pH 3.15)
Mobile Phase B: Acetonitrile
Mobile Phase Ratio: 50: 50 (A: B)
Flow Rate: 0.6 mL/min
Column Temperature: 35 °C
Injection Volume: 20 µL
Detector: ELSD

Representative Chromatogram from Lot: LRAD8501 Impurities Analysis



Impurities Detected:

Total Impurities: **None**

LOSS ON DRYING/VOLATILES

Method:

Sample Size: 500 mg

Mean of three measurements, Loss = **0.046%**

Certification process details:

The certified purity is determined by qNMR and calculated as

$$P_{\text{Sample}} = \frac{I_{\text{Analyte}}}{I_{\text{CRM}}} \cdot \frac{N_{\text{CRM}}}{N_{\text{Analyte}}} \cdot \frac{M_{\text{Analyte}}}{M_{\text{CRM}}} \cdot \frac{m_{\text{CRM}}}{m_{\text{Sample}}} \cdot P_{\text{CRM}}$$

- *P* Sample Purity of samples as mass fraction (%)
- *P* CRM Purity of CRM as mass fraction (%)
- *I* Analyte Integral of the analyte signal
- *I* CRM Integral of CRM signal
- *N* Analyte Number of analyte nuclei
- *N* CRM Number of CRM nuclei
- *M* Analyte Molecular mass of the analyte (g/mol)
- *M* CRM Molecular mass of the CRM (g/mol)
- *m* Sample Mass of sample (mg)
- *m* CRM Mass of CRM (mg)

METHOD: quantitative NMR spectroscopy

Condition: Bruker 500 MHz

Solvent: D2O_NaOD

Internal standard(CRM): Potassium phthalate monobasic, MilliporeSigma 14659 lot: BCCK7014

CERTIFIED PURITY BY qNMR (Mass Fraction, n = 9)

99.2 % U_{CRM} = ± 0.4 %, k = 2.0 (qNMR / as is basis)

Homogeneity assessment:

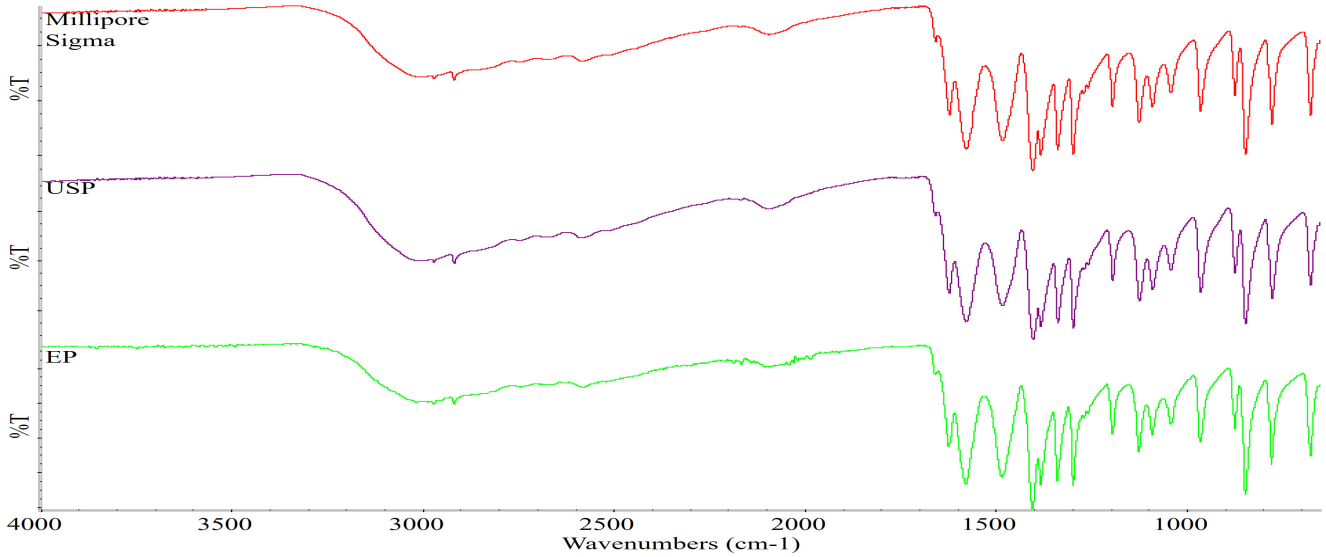
Homogeneity was assessed in accordance with ISO Guide 35. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared by Single Factor Analysis of Variance (ANOVA). The uncertainty due to homogeneity was derived from the ANOVA. Heterogeneity was not detected under the conditions of the ANOVA.
Analytical method: QNMR
Sample size: 15 mg

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.

Identification Test:

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

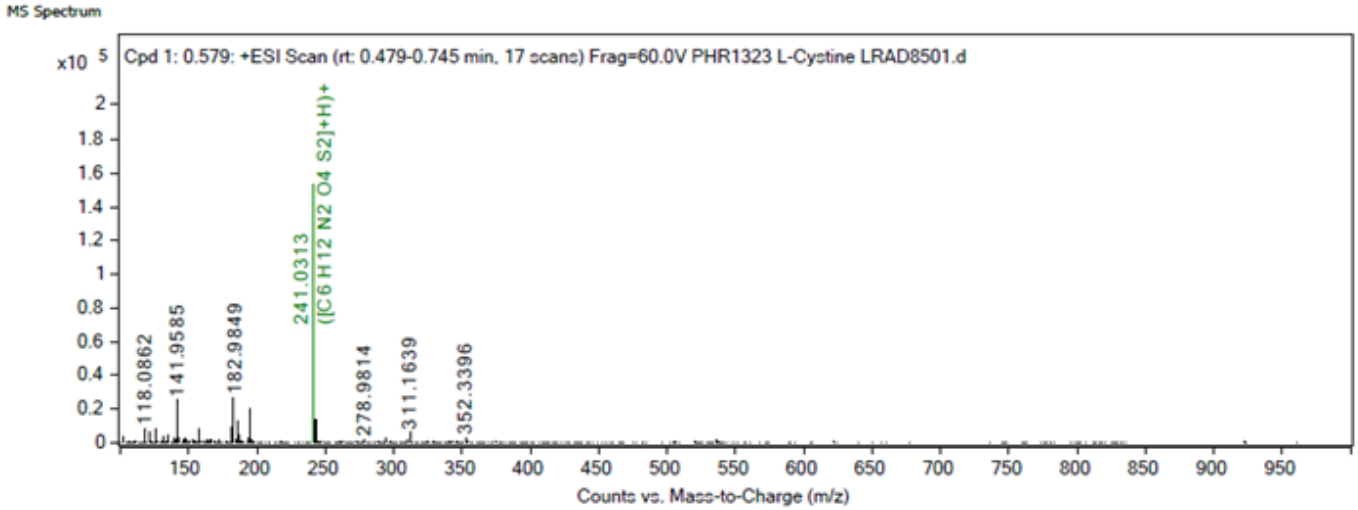


MilliporeSigma Lot: LRAD8501

USP 1161586 LOT: R19610

EP C3300000 BATCH: 3.0

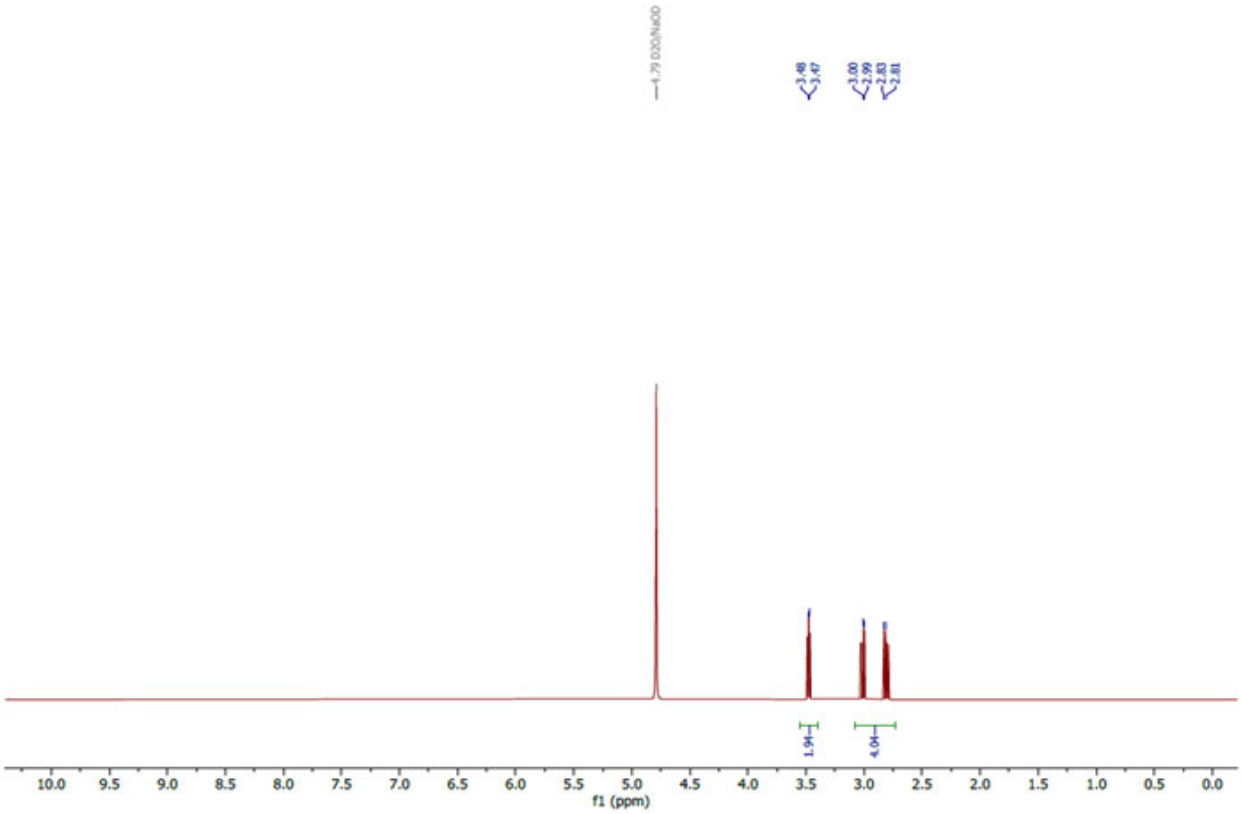
MASS SPECTRUM
Method: HR-QTOF; 4.0 kV ESI+; temperature: 325 °C



Theoretical value: 241.0317 *m/z*

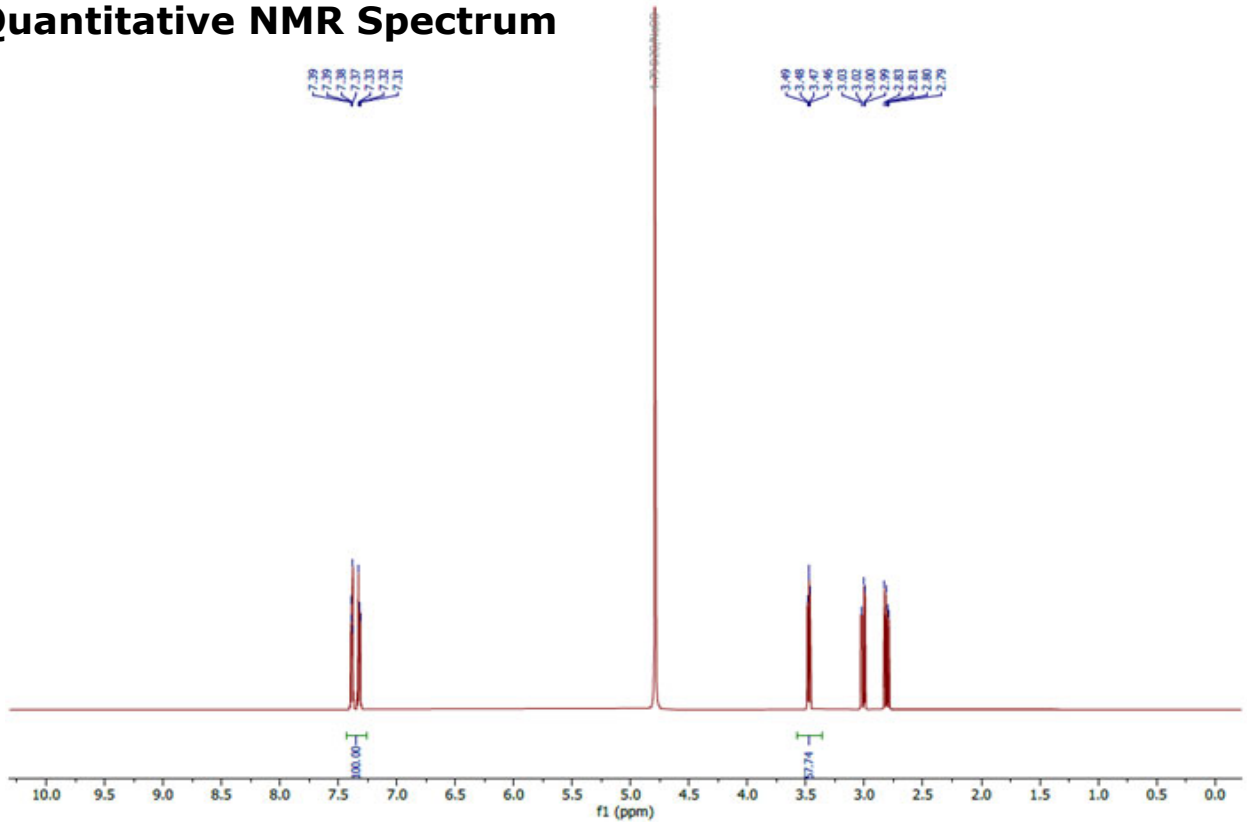
The signal of the MS spectrum is consistent with the theoretical value and its interpretation is consistent with the structural formula.

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



Consistent with structure

Quantitative NMR Spectrum



OPTICAL ROTATION

Specification: -215° to -225°
Perkin Elmer Polarimeter 343
Wavelength: 589 nm
Concentration: 200 mg / 10 mL
Cell Path: 100 mm
Mean of three measurements = **-223.72 °**

Certificate of analysis revision history:

Certificate version	Date	Reason for version
LRAD8501.01	22 AUG 2024	Original release date