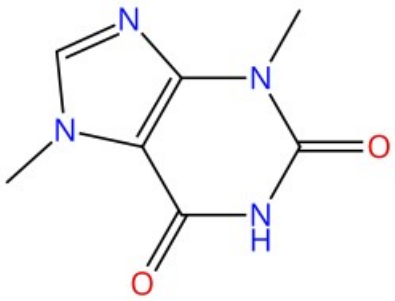
Certificate of Analysis - Certified Reference Material

Theobromine

Product no.: PHR1837-100MG
Lot no.: **LRAD9964**
Description of CRM: White powder
Expiry date: February 2029
Storage: REFRIGERATE (2 °C to 8 °C)
Certificate version: LRAD9964.02 *(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: C7H8N4O2
Molecular mass: 180.17
CAS No. 83-67-0



Analyte	Purity (certification method / basis)
Theobromine	**99.6 % U_{CRM} = ± 0.3 , k = 2.00 (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: 20 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: 30 April 2025

[Andy Ommen - QC Authority]

[Christopher Rucinski - QA Authority]

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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: 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. 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 100 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 chromatographic identification analysis demonstrates direct traceability to Pharmacopeial standards through compendial method analysis)

TRACEABILITY COMPARISON vs. USP REFERENCE STANDARD 1086064 (as is basis)

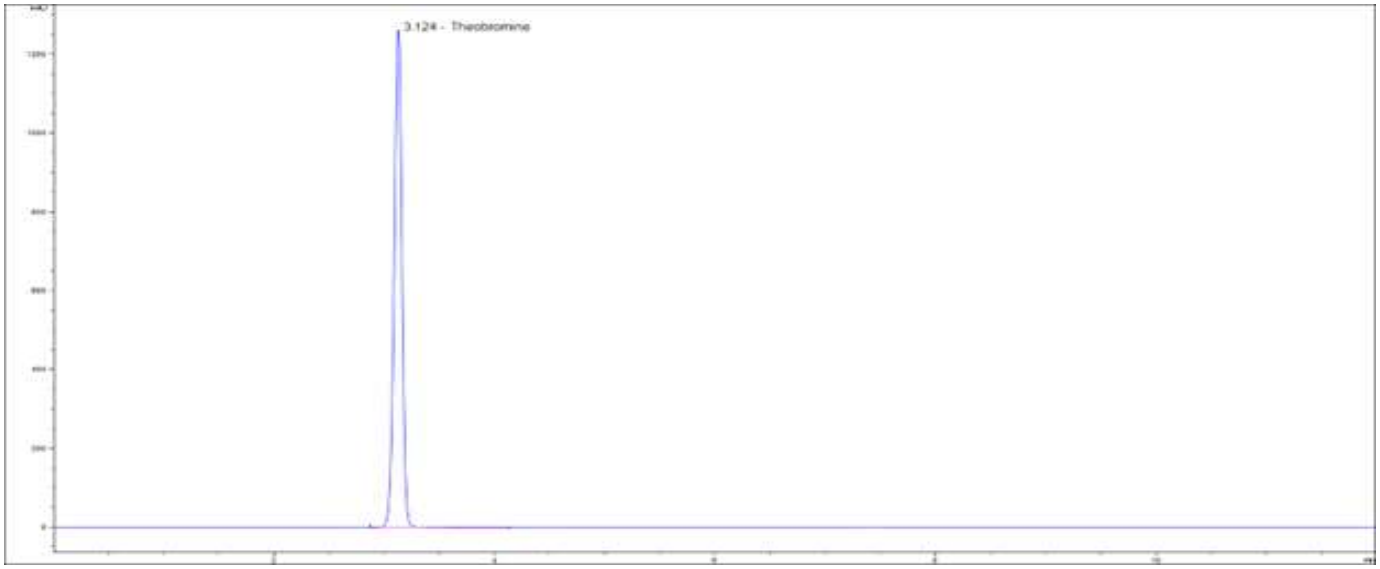
MilliporeSigma Lot LRAD9964 vs. USP Lot F212M0

METHOD: HPLC (In House MV# 165)

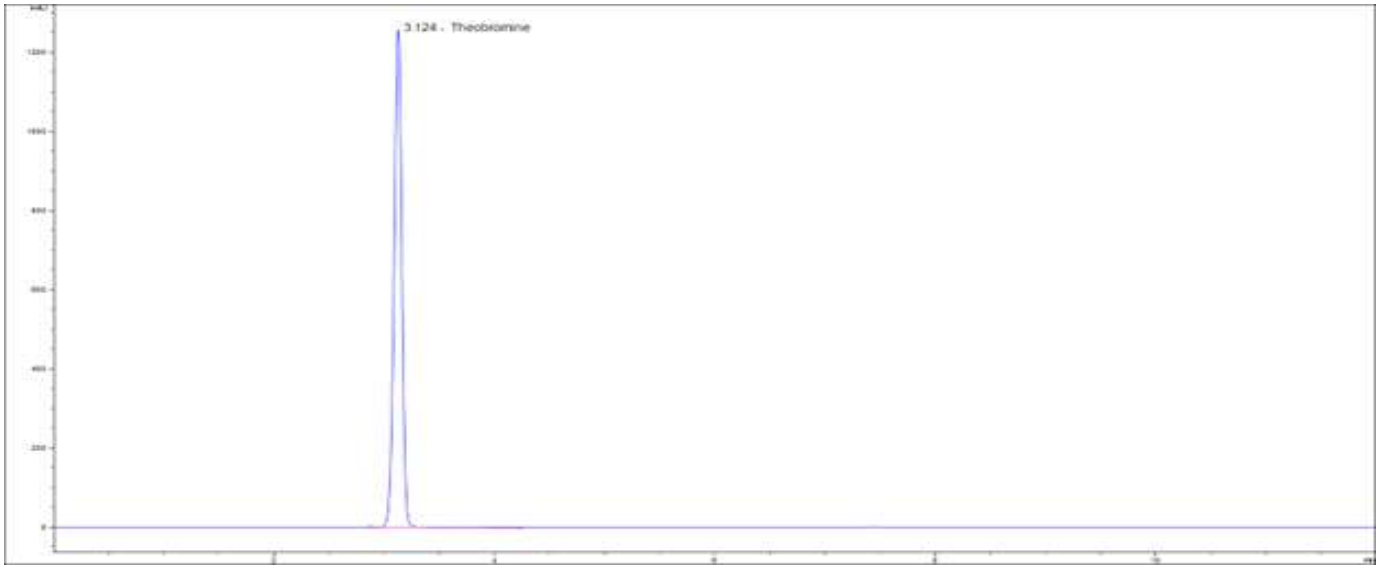
Column: Supelcosil-LC-18-DB, 15 cm X 4.6 mm, 5 µm particle size
Mobile Phase A: 0.82 g/L sodium acetate in water (pH 4.5)
Mobile Phase B: Acetonitrile
Mobile Phase C: Tetrahydrofuran
Mobile Phase Ratio: A:B:C, 955:25:20
Flow Rate: 1 mL/min
Column Temperature: 30 °C
Injection Volume: 10 µL
Detector: DAD

Wavelength: 275 nm

Representative Chromatogram from MilliporeSigma Lot:LRAD9964 Analysis



Representative Chromatogram from USP 1086064 Lot: F212M0 Analysis

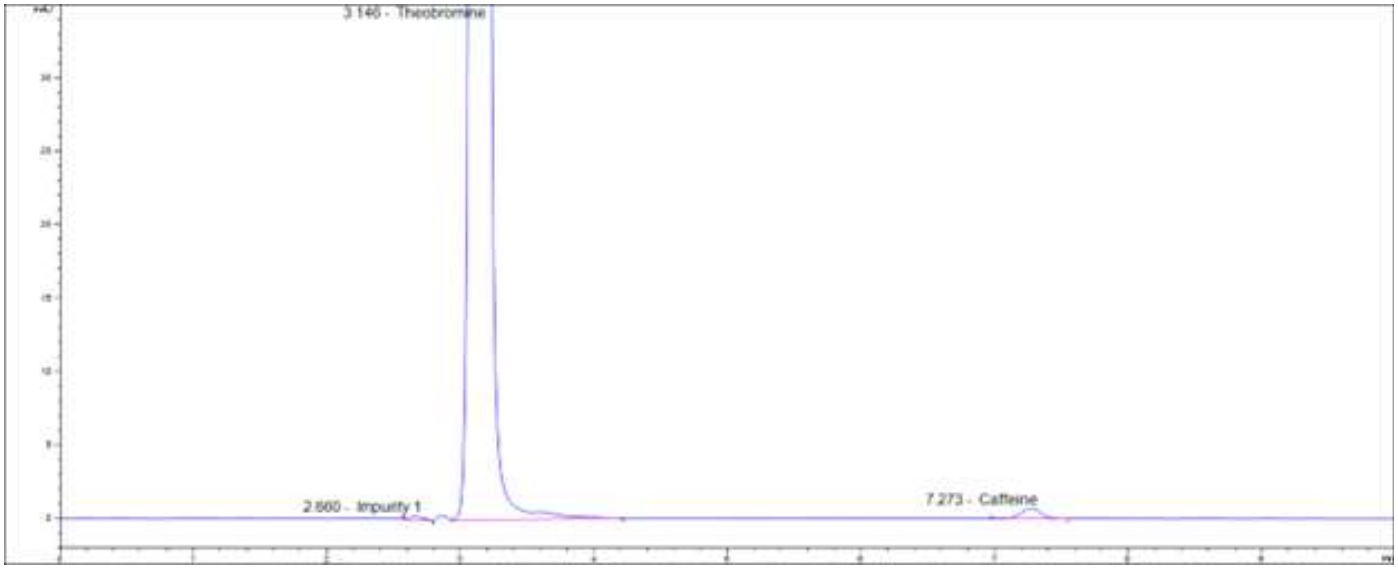


CHROMATOGRAPHIC IMPURITY ANALYSIS

METHOD: HPLC (In House MV# 165)

Column: Supelcosil-LC-18-DB, 15 cm X 4.6 mm, 5µm particle size
Mobile Phase A: 0.82 g/L sodium acetate in water (pH 4.5)
Mobile Phase B: Acetonitrile
Mobile Phase C: Tetrahydrofuran
Mobile Phase Ratio: A:B:C, 955:25:20
Flow Rate: 1 mL/min
Column Temperature: 30 °C
Injection Volume: 10 µL
Detector: DAD
Wavelength: 275 nm

Representative Chromatogram from Lot: LRAD9964 Impurities Analysis



Impurities Detected:

Impurity 1:	0.055 %
Caffeine:	0.117 %
Total Impurities:	**0.172** %

LOSS ON DRYING/VOLATILES
Method: In house method
Sample Size: 100 mg
Mean of three measurements, Loss = **None**

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): Potasium hydrogene phthalate Product No. 14659 lot BCCK7014

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

99.6 % U_{CRM} = ± 0.3 %, k = 2.00 (qNMR / as is basis)

Homogeneity assessment:

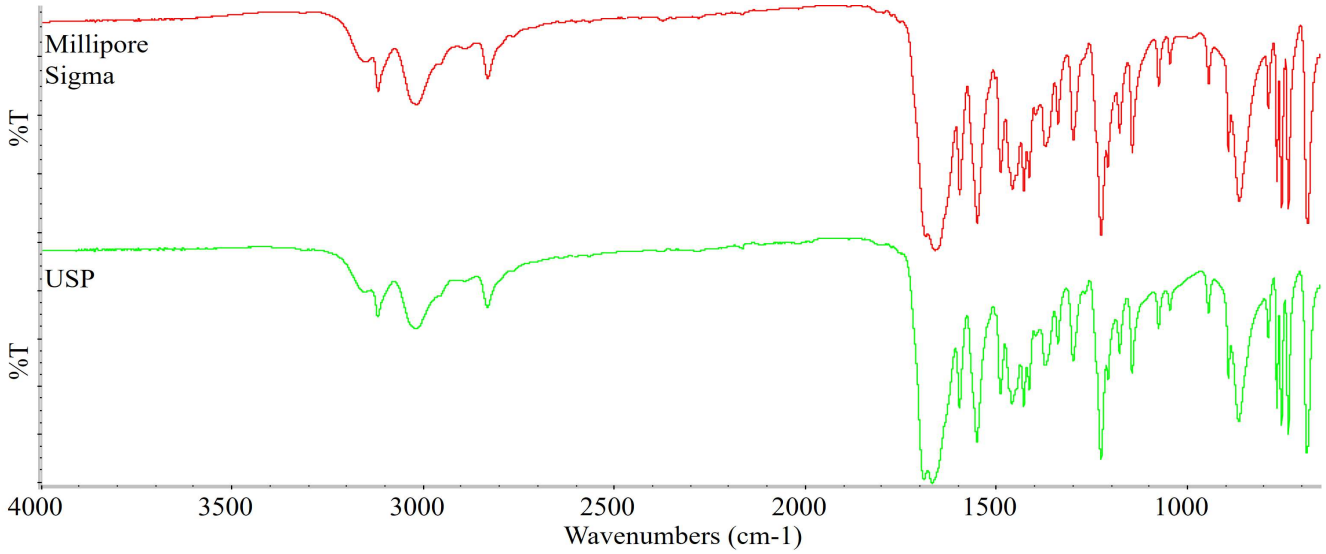
Homogeneity was assessed in accordance with ISO Guide 35. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared by Single Factor Analysis of Variance (ANOVA). The uncertainty due to homogeneity was derived from the ANOVA. Heterogeneity was not detected under the conditions of the ANOVA.
Analytical method: QNMR
Sample size: 20 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:

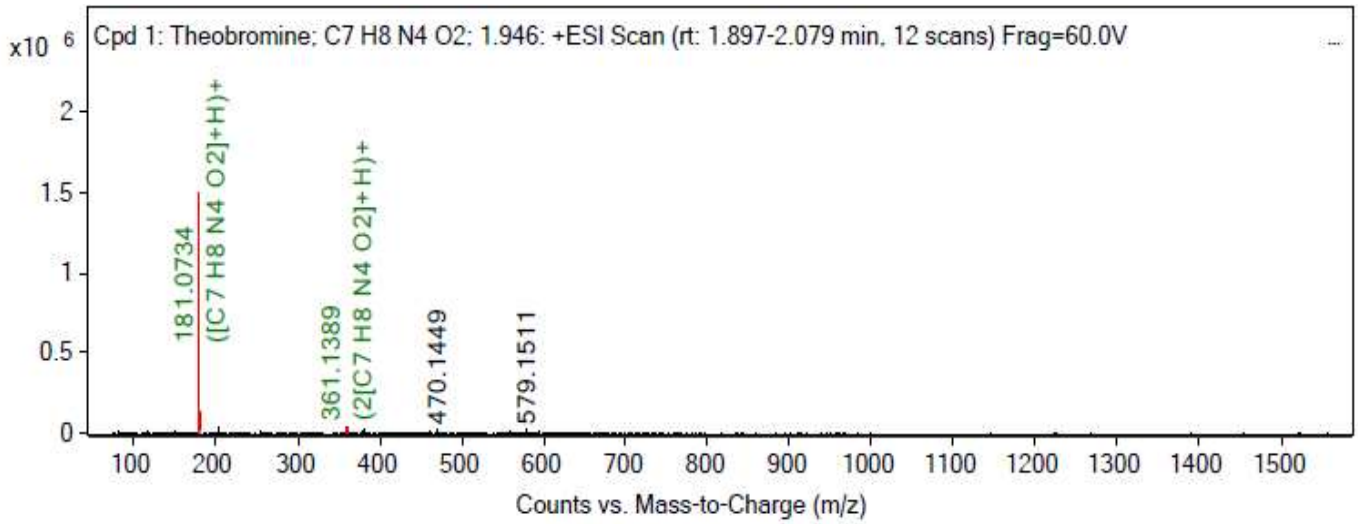
INFRARED SPECTROPHOTOMETRY (Comparative identification analysis demonstrates direct traceability to Pharmacopeial standards)



MilliporeSigma Lot: LRAD9964

USP 1086064 LOT: F212M0

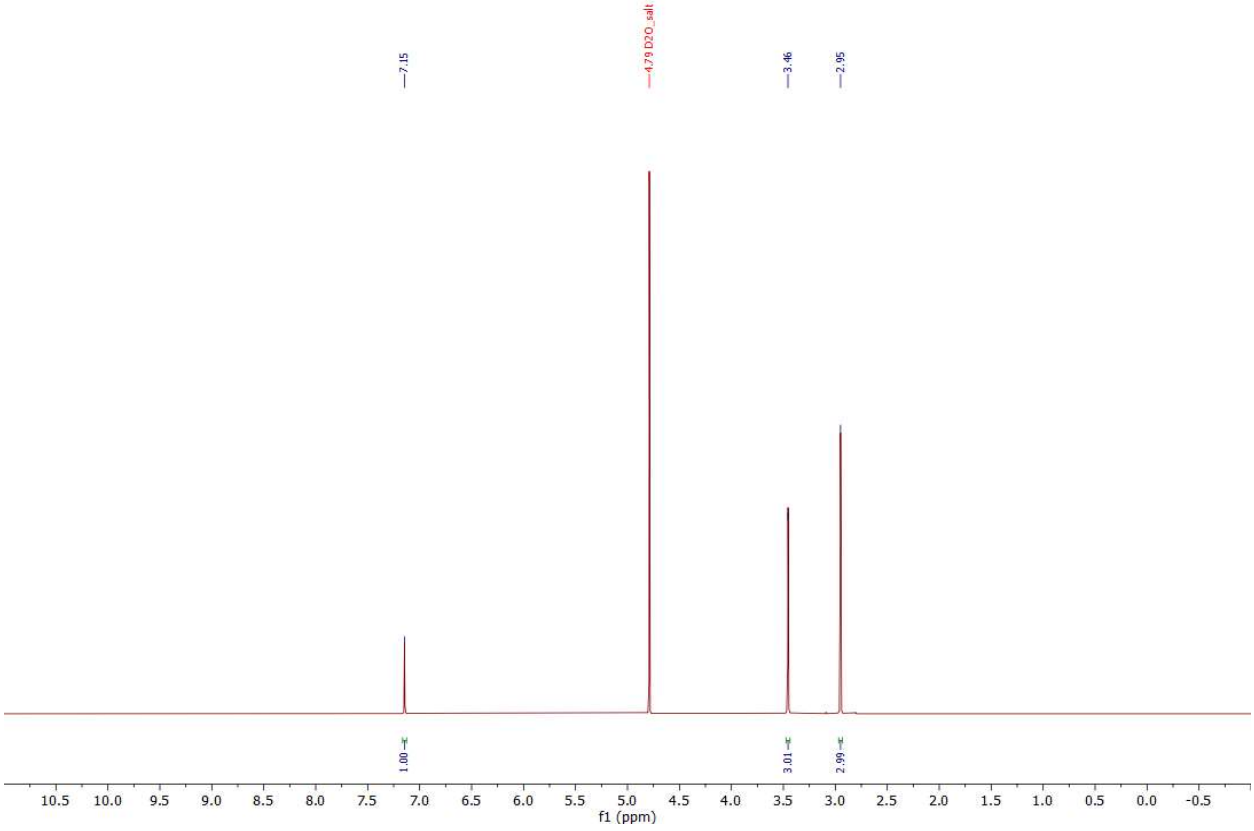
MASS SPECTRUM
Method: HR-QTOF; 4.0 kV ESI+; temperature: 325 °C



Theoretical value: 181.0725 *m/z*

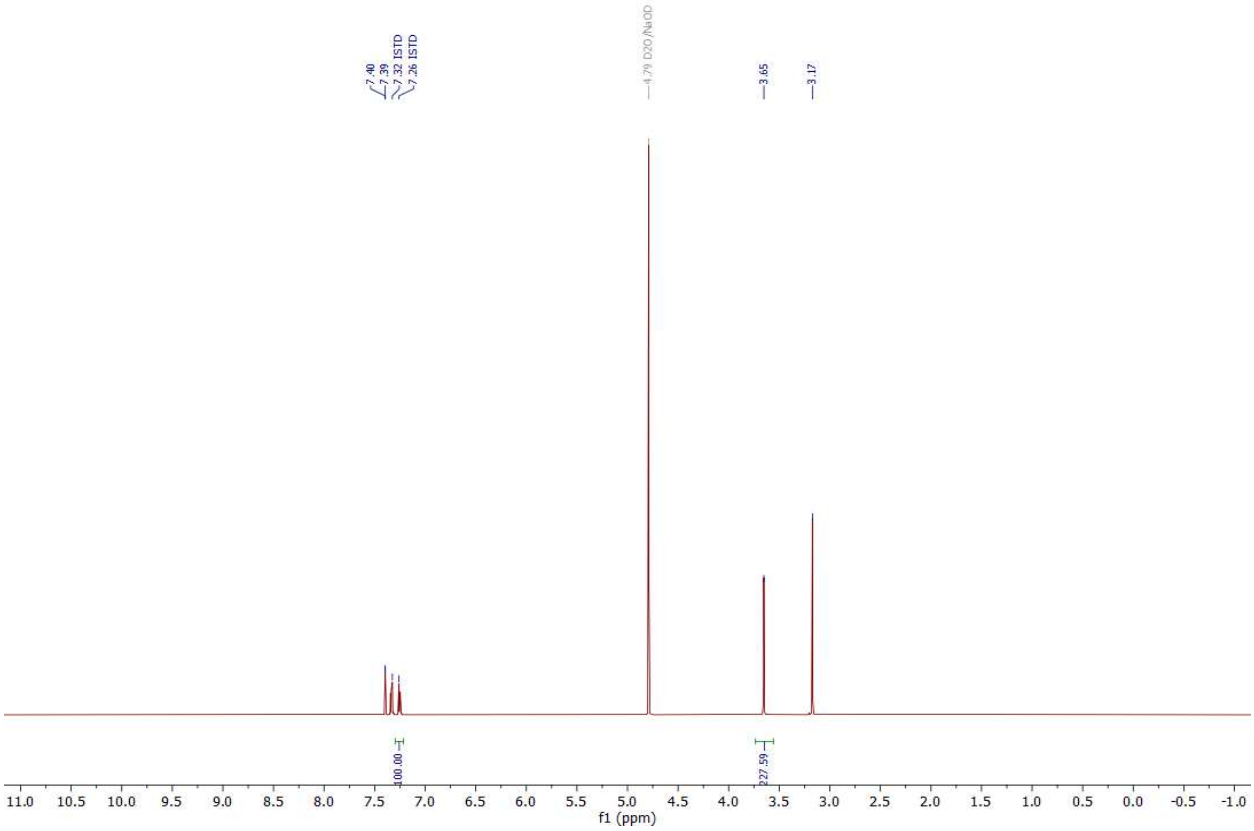
The signal of the MS spectrum is consistent with the theoretical value and its interpretation is consistent with the structural formula.

1H NMR Qualitative Spectrum



Consistent with structure

Quantitative NMR Spectrum



Certificate of analysis revision history:

Certificate version	Date	Reason for version
LRAD9964.01	24 FEB 2025	Original release date
LRAD9964.02	30 April 2025	Addition of USP Traceability