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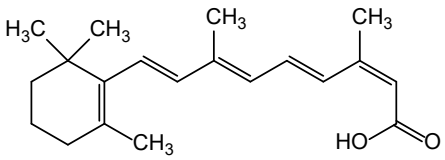
Certificate of Analysis – Certified Reference Material

ISOTRETINOIN



Product no.:	PHR1188-3x100MG
Lot no.:	LRAD4514
Description of CRM:	Dark Orange Powder
Expiry date:	30 June 2027
Storage:	-25 °C to -10 °C Protect from Light and Air
Certificate version:	LRAD4514.1 *(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.)*
Chemical formula:	$C_{20}H_{28}O_2$
Molecular mass:	300.4
CAS No.:	4759-48-2

Analyte	Certified Purity ± associated uncertainty *U*, *U=k · u (k=)* (qNMR/ basis)
ISOTRETINOIN	**98.0 % Ucrm = ±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. 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 "Certification process details" on page 3.
Intended use:	Intended for R&D and Analytical Use only. Not for drug, household or other uses
Minimum sample size:	15 mg
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. Attachment of a 20 mm aluminum crimp seal recommended for unused portions.
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:	23 June 2023

ANAB ACCREDITED ISO 17034 REFERENCE MATERIAL

ISO 17034
AR-1470

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Sigma-Aldrich RTC is a subsidiary of Merck KGaA, Darmstadt, Germany.

Packaging: 100 mg in amber ampule

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. 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.

Traceability Assay:
Comparative assay demonstrates direct traceability to Pharmacopeial Standards

ASSAY vs. USP REFERENCE STANDARD (1353500) (as is basis)

ASSAY VALUE	**vs. USP LOT**
97.2 %	**R09860** Labeled Content = 0.999 mg/mg

ASSAY vs. EP CRS (I0800000) (as is basis)

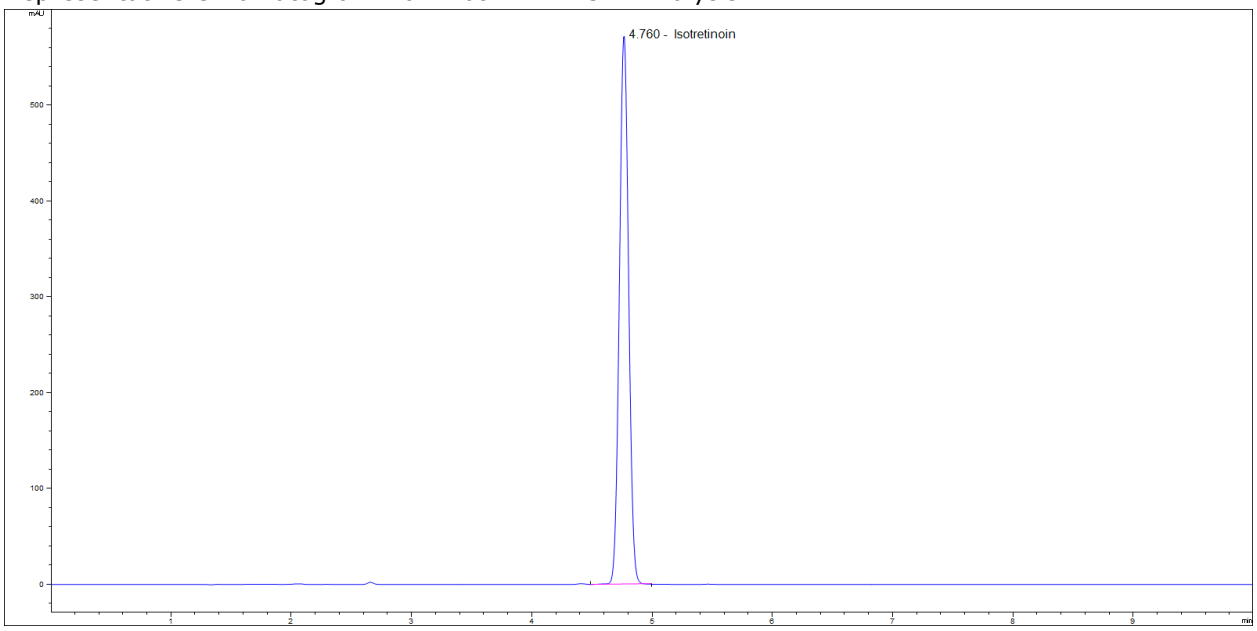
ASSAY VALUE	**vs. EP BATCH**
97.2 %	**6.0** Labeled Content = None Assigned Content = 67.2 %*

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

Method: HPLC (ref.: Adapted from Isotretinoin Capsules, Current Compendial Monographs)

Column: Ascentis Express C18, 250 mm x 4.6mm, 5µm particle size
Mobile Phase A: 0.5% Acetic Acid in Water
Mobile Phase B: 0.5% Acetic Acid in Methanol
Mobile Phase Ratio: (10:90) (A: B)
Flow Rate: 1.5 mL/min
Column Temperature: 30 °C
Injection Volume: 5 µL
Detector: DAD, Wavelength: 353 nm

Representative Chromatogram from Lot: LRAD4514 Analysis



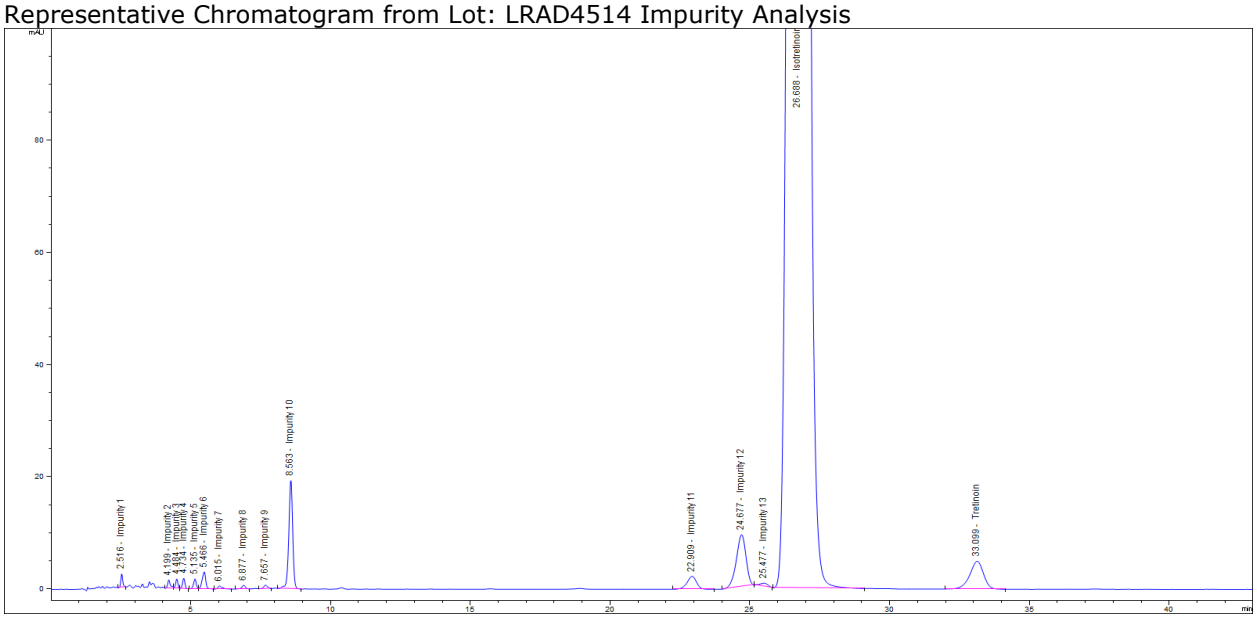
CHROMATOGRAPHIC IMPURITY ANALYSIS
METHOD: HPLC (ref.: , Current Compendial Monographs)
Column: Ascentis Express C18, 150 mm x 4.6mm, 2.7µm particle size
Mobile Phase: (Methanol:Water:Acetic Acid)
Mobile Phase Ratio: (770:225:5)
Flow Rate: 1.0 mL/min
Column Temperature: 30 °C
Injection Volume: 3 µL
Detector: DAD, Wavelength: 355 nm

Impurities Detected:

Impurity 1:	0.027 %	Impurity 2:	0.021 %
Impurity 3:	0.020 %	Impurity 4:	0.024 %
Impurity 5:	0.027 %	Impurity 6:	0.063 %
Impurity 7:	0.013 %	Impurity 8:	0.014 %
Impurity 9:	0.021 %	Impurity 10:	0.578 %
Impurity 11:	0.123 %	Impurity 12:	0.520 %
Impurity 13:	0.024 %	Tretinoin:	0.393 %

Total Impurities: **1.87 %**

Representative Chromatogram from Lot: LRAD4514 Impurity Analysis



RESIDUAL SOLVENTS
Method: GC-MS Headspace (ref.: Adapted from Residual Solvents USP <467>)
Column: SPB-624, 30 m x 0.25 mm x 1.4 µm
Carrier gas: He
Flow: 1.0 mL/min
Split Ratio: 5:1
Injection/Temperature: 1 mL/180 °C
Temperature Program: 40 °C for 5 min, 8 °C/min to 200 °C, hold 5 min

Solvents Detected:

Ethyl Acetate: 0.18 %

LOSS ON DRYING/VOLATILES
Method: Under Vacuum at Room Temperature for 16 hours (ref.: Current Compendial Monographs)
Mean of three measurements, Loss = **None**

RESIDUE ANALYSIS
Method: Sulfated Ash (ref.: Current Compendial Monographs)
Sample Size: ~ 100 mg
Mean of three measurements, Residue = **0.09 %**

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)

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

98.0 % U_{crm} = ± .04 %, k = 2.0
(as is basis)

METHOD: quantitative NMR spectroscopy

Condition: Bruker 500 MHz

Solvent: DMSO-d_6

Internal standard: Ethylene carbonate (*Trace*CERT 01380)

Homogeneity assessment: Homogeneity was assessed in accordance with ISO Guide 35. The material is tested by qNMR measurements using 4 or 9 subsamples which are taken from different positions in the entire bulk material. The recommended minimal sample size is taken for all the homogeneity test samples. Analysis of variance (ANOVA) result are included into the calculation of content uncertainty of this CRM.

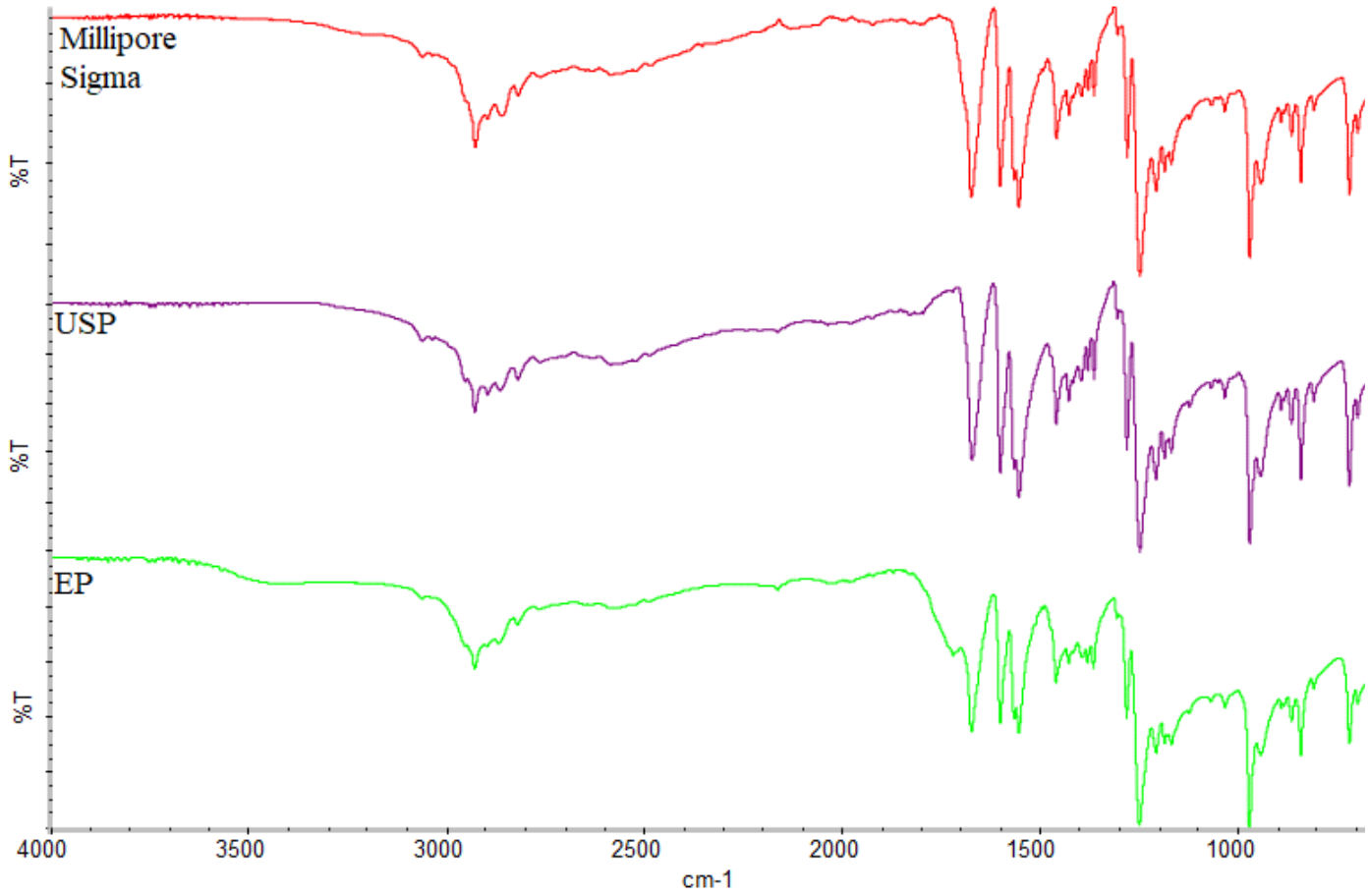
Analytical method: qNMR Sample size: 15 mg

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)

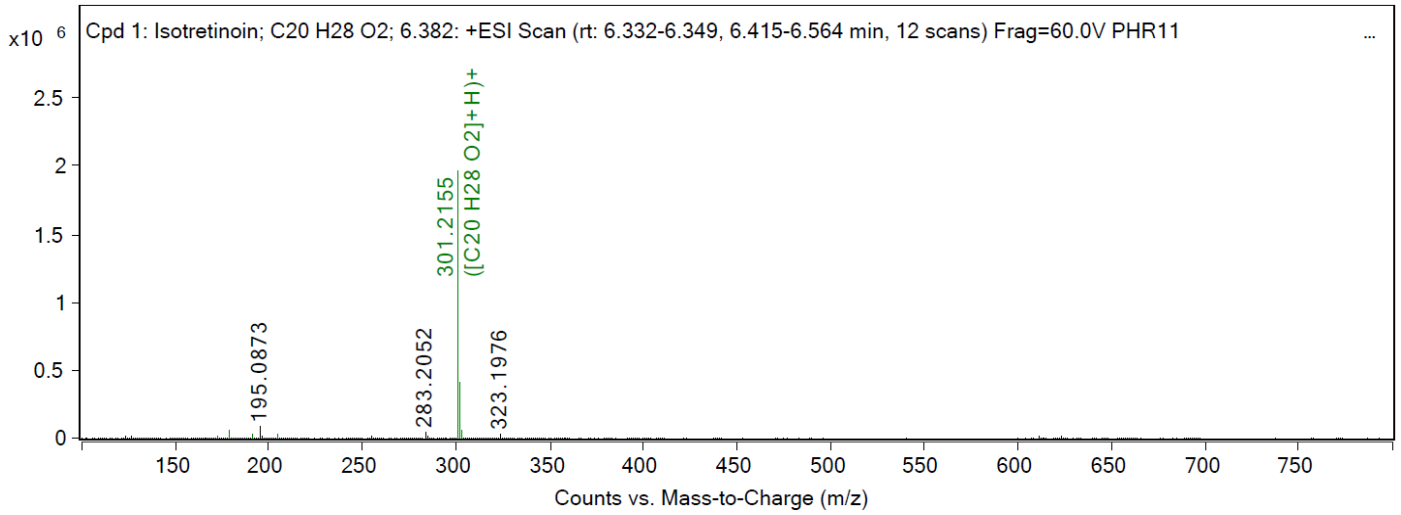


MilliporeSigma Lot: LRAD4514 vs USP Lot: R09860 / EP Batch: 6.0

Indicative Values:

MASS SPECTRUM

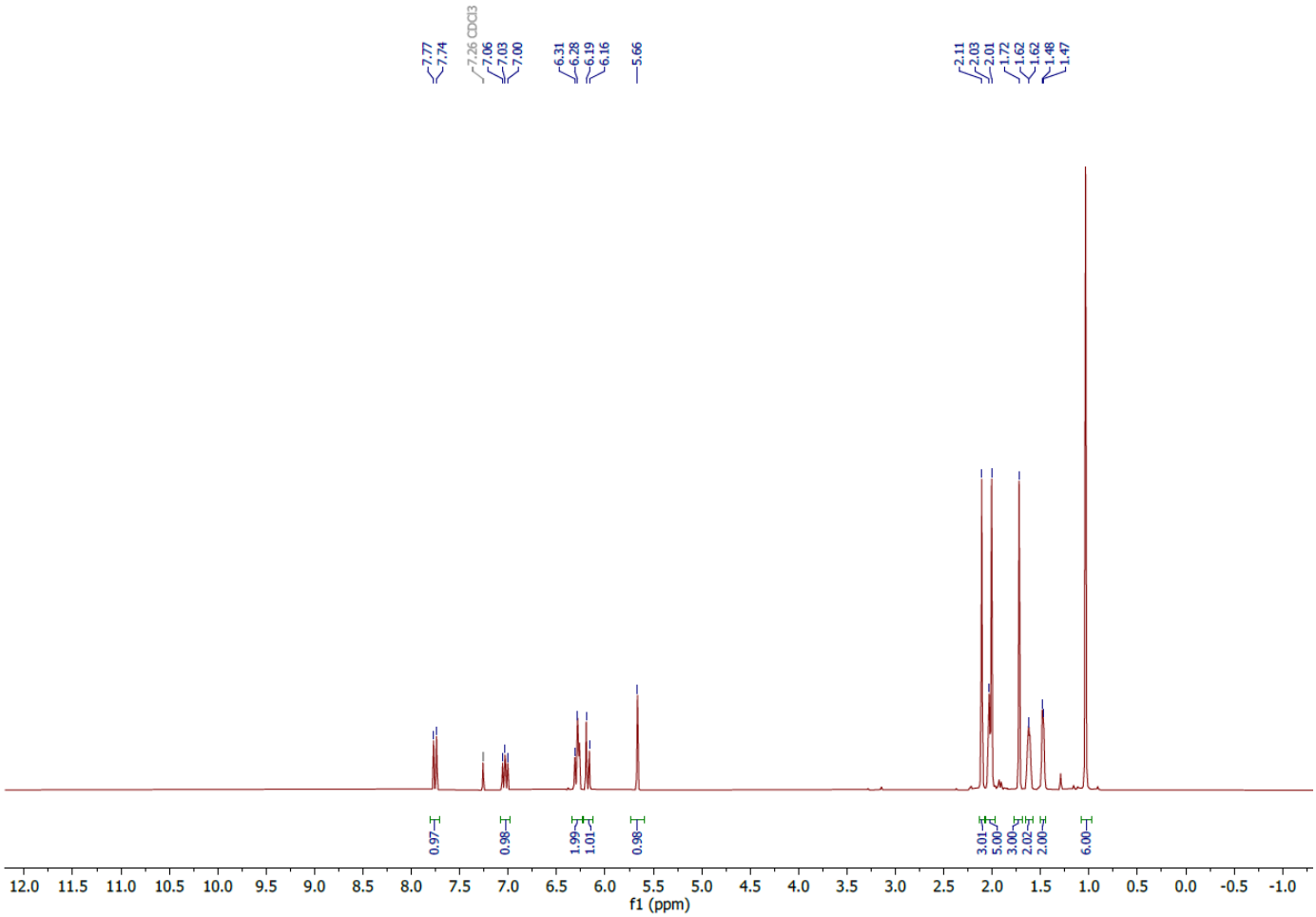
Method: HR-QTOF; 4.0 kV ESI+; temperature: 325 °C



Theoretical value: 301.2167 m/z

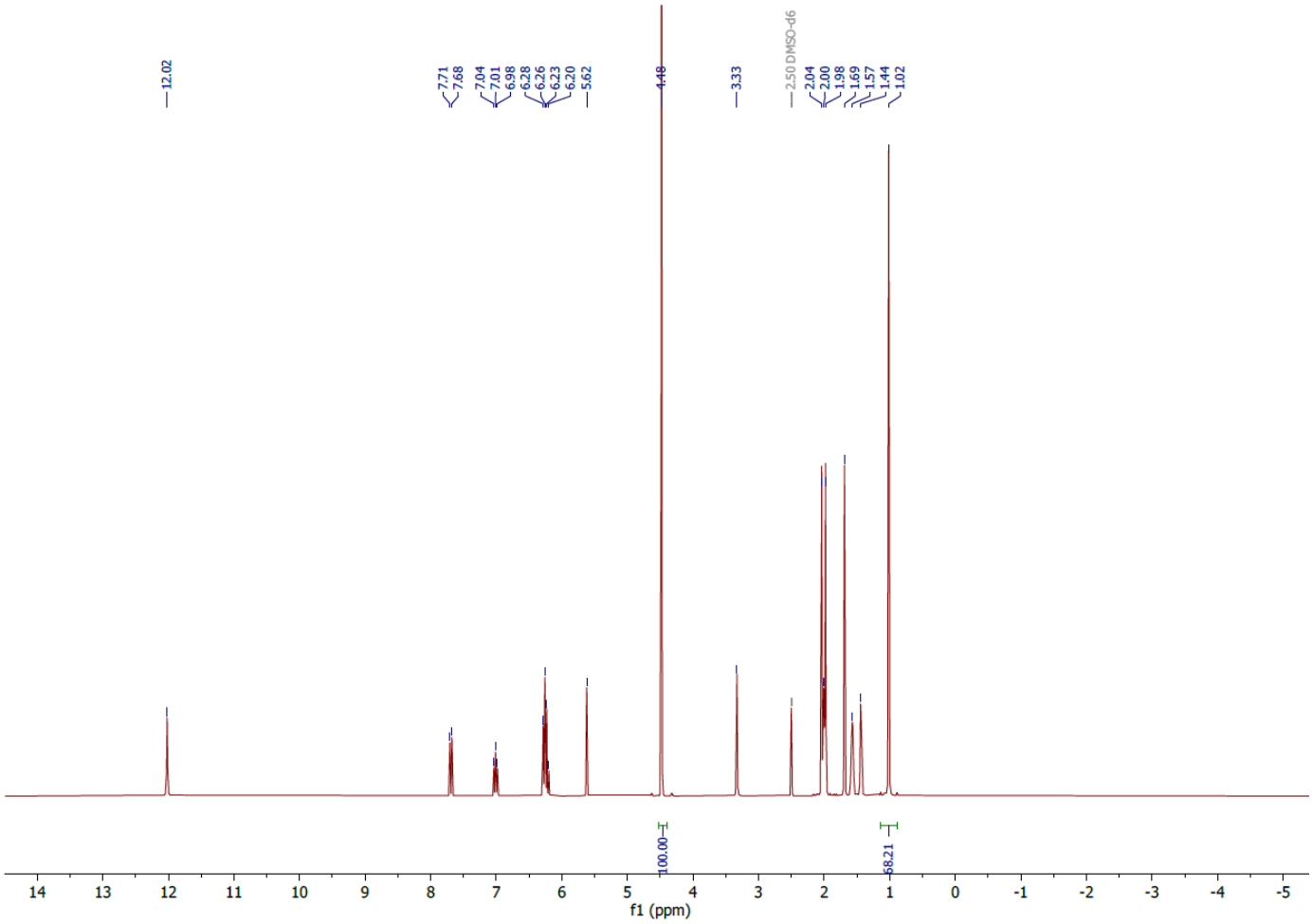
The signal of the MS spectrum is consistent with the theoretical value and its interpretation is consistent with the structural formula.

^{1}H NMR



Consistent with structure

Quantitative NMR Spectrum



Certificate of analysis revision history:

Certificate version	Date	Reason for version
LRAD4514.1	23 June 2023	Original Release

Disclaimer:

The purchaser is required to determine the suitability of this product for any particular application. Sigma-Aldrich RTC makes no warranty of any kind, express or implied, other than its products meet all quality control standards set by Sigma-Aldrich RTC. We do not guarantee that the product can be used for any particular application.