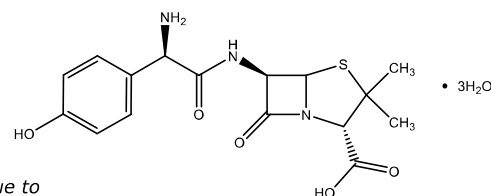


Certificate of Analysis – Certified Reference Material

AMOXICILLIN TRIHYDRATE

Product no.: PHR1127-1G
Lot no.: LRAB3723
Description of CRM: White Powder
Expiry date: 31 December 2022
Storage: Freezer/Protect from Light
Certificate version: LRAB3723,3 (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: C₁₆H₁₉N₃O₅S·3H₂O
Molecular mass: 419.45
CAS No.: 61336-70-7



Analyte	Certified Purity ± associated uncertainty U, U=k · u (k=) (Mass Balance/basis)
Amoxicillin Trihydrate	85.2 % U _{crm} = ±0.35%, k = 2.3 (As Amoxicillin, 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: 60 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: 31 March 2020



[Andy Ommen; Quality Control]

[Mark Pooler; Quality Assurance]



Packaging:

1G in amber vial

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:**Method: HPLC (ref.: Amoxicillin, Current Compendial Monographs)****ASSAY vs. USP REFERENCE STANDARD (as is basis)****ASSAY VALUE****86.2 %****vs. USP LOT****R106H0**

Labeled Content = 866 µg/mg, As Amoxicillin

Column: LiChrosphere RP-18 4.6 x 250 mm, 5 µm particle size

Mobile Phase: 6.8 g/L Monobasic potassium phosphate in Water (pH 5.0), Acetonitrile (96:4)

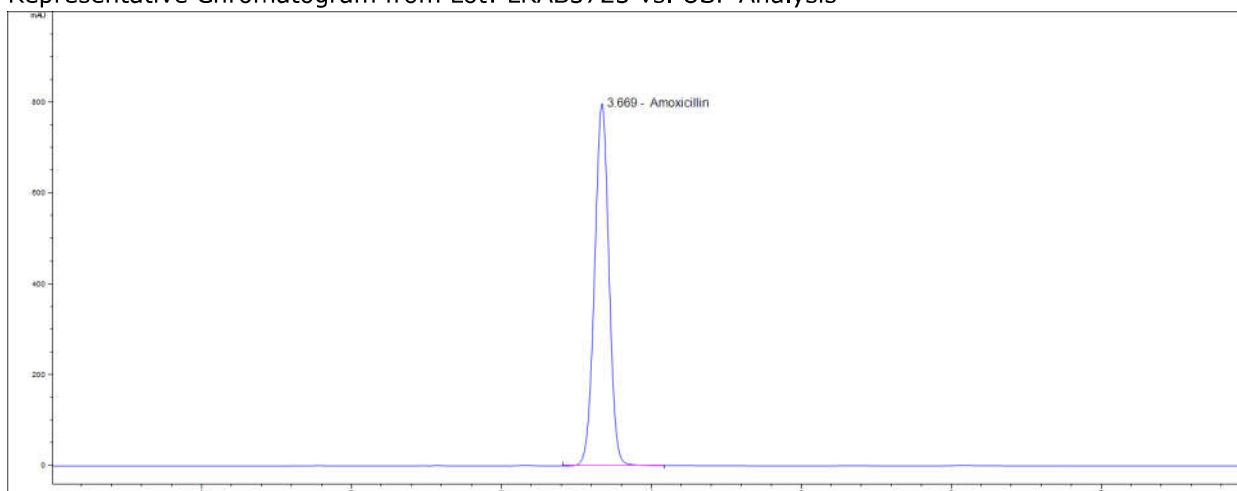
Flow Rate: 1.5 mL/min

Column Temperature: 30 °C

Injection Volume: 5 µL

Detector: DAD, Wavelength: 230 nm

Representative Chromatogram from Lot: LRAB3723 vs. USP Analysis

**ASSAY vs. EP CRS (as is basis)****ASSAY VALUE****86.8%****vs. EP BATCH****6.0**

Labeled Content = 85.9 %, As Amoxicillin

ASSAY vs. BP CRS (as is basis)

ASSAY VALUE

87.4 %

vs. BP BATCH

4021

Labeled Content = 86.5 %, As Amoxicillin

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

Mobile Phase: 6.8 g/L Monobasic potassium phosphate in Water (pH 5.02); acetonitrile (24:1)

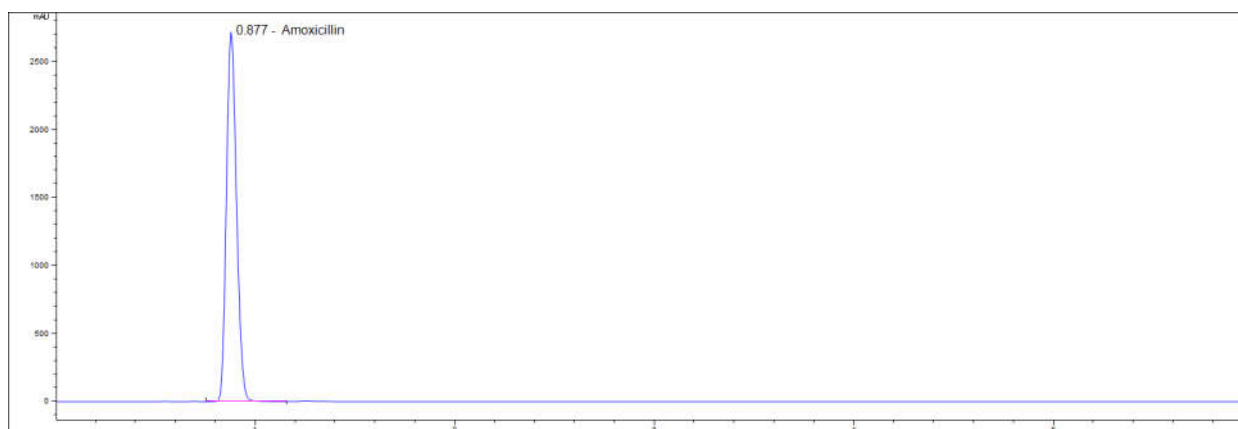
Flow: 1.5 mL/min

Column Temperature: 30 °C

Injection: 10 µL

Detector: 230 nm

Representative Chromatogram from Lot: LRAB3723 vs. EP/BP Analysis



Certification process details:

The certified purity is determined by mass balance and calculated as

$$\% \text{ Purity} = \left(\frac{(100 - TCI)}{100} * \frac{(100 - LOD)}{100} * \frac{(100 - H2O)}{100} * \frac{(100 - RS)}{100} \right) * 100\%$$

- TCI = Total Chromatographic Impurities
- LOD = Loss on Drying
- H₂O = Water content determined by Karl Fischer analysis
- ROI = Residue on Ignition
- RS = Residual Solvents

Methods for impurity determination may be added or deleted as required. The following techniques are applied:

CHROMATOGRAPHIC IMPURITY ANALYSIS

METHOD: HPLC (ref.: Amoxicillin Trihydrate,, Current Compendial Monographs)

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

Mobile Phase A: Buffer

Mobile Phase B: Methanol

Buffer Solution: 2.72 g/L Potassium dihydrogen phosphate in Water (pH 5.11)

Gradient:

Time (min)	% Mobile Phase A	% Mobile Phase B
0-10	97	3
10 - 22	97 → 75	3 → 25
22 - 26	75 → 97	25 → 3

Column Temperature: 40°C

Flow: 1.5 mL/min

Injection: 10 µL

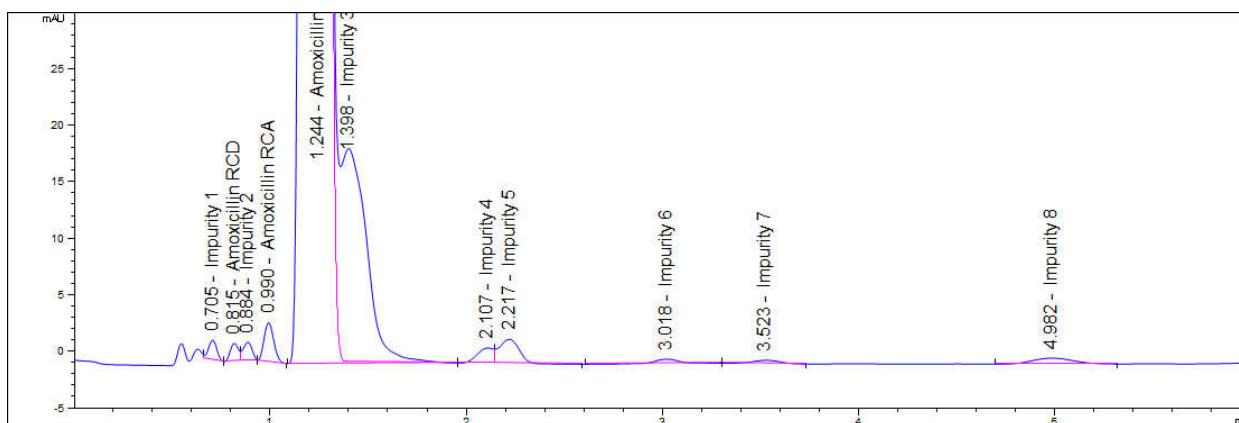
Detector: 210 nm

Impurities Detected:

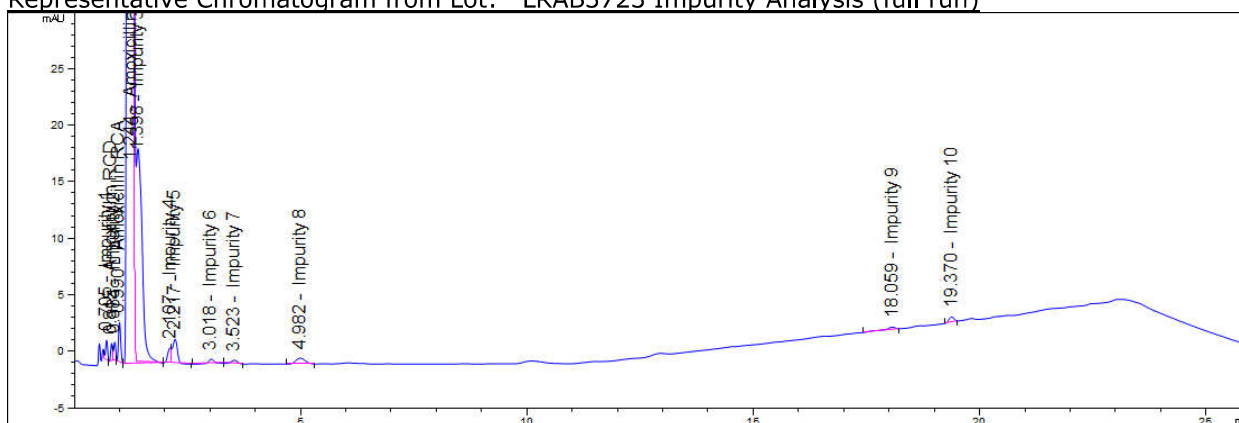
Impurity 1:	0.04 %	Amoxicillin RCD:	0.04 %
Impurity 2:	0.04 %	Amoxicillin RCA:	0.1 %
Impurity 3:	1.5 %	Impurity 4:	0.06 %
Impurity 5:	0.15 %	Impurity 6:	0.03 %
Impurity 7:	0.02 %	Impurity 8:	0.06%
Impurity 9:	0.02 %	Impurity 10:	0.03 %

Total Impurities: **2.1 %**

Representative Chromatogram from Lot: LRA3723 Impurity Analysis (early impurities)



Representative Chromatogram from Lot: LRA3723 Impurity Analysis (full run)



RESIDUAL SOLVENTS

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

Column: SPB-624

Carrier gas: He

Flow: 1.2 mL/min

Split Ratio: 1:5

Injection/Temperature: 1 mL/250 °C

Temperature Program: 40 °C for 8 min, 8 °C/min to 200 °C, hold 8 min

Solvents Detected: None

WATER DETERMINATION

Method: Karl Fischer (ref.: Current Compendial Monographs)

Mean of three measurements, Water Content = **12.9 %**

RESIDUE ANALYSIS

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

Sample Size: ~ 1 g

Mean of three measurements, Residue = **0.07%**

CERTIFIED PURITY BY MASS BALANCE

85.2 % $U_{\text{crm}} = \pm 0.35\%$, $k = 2.3$
(As Amoxicillin, 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: HPLC

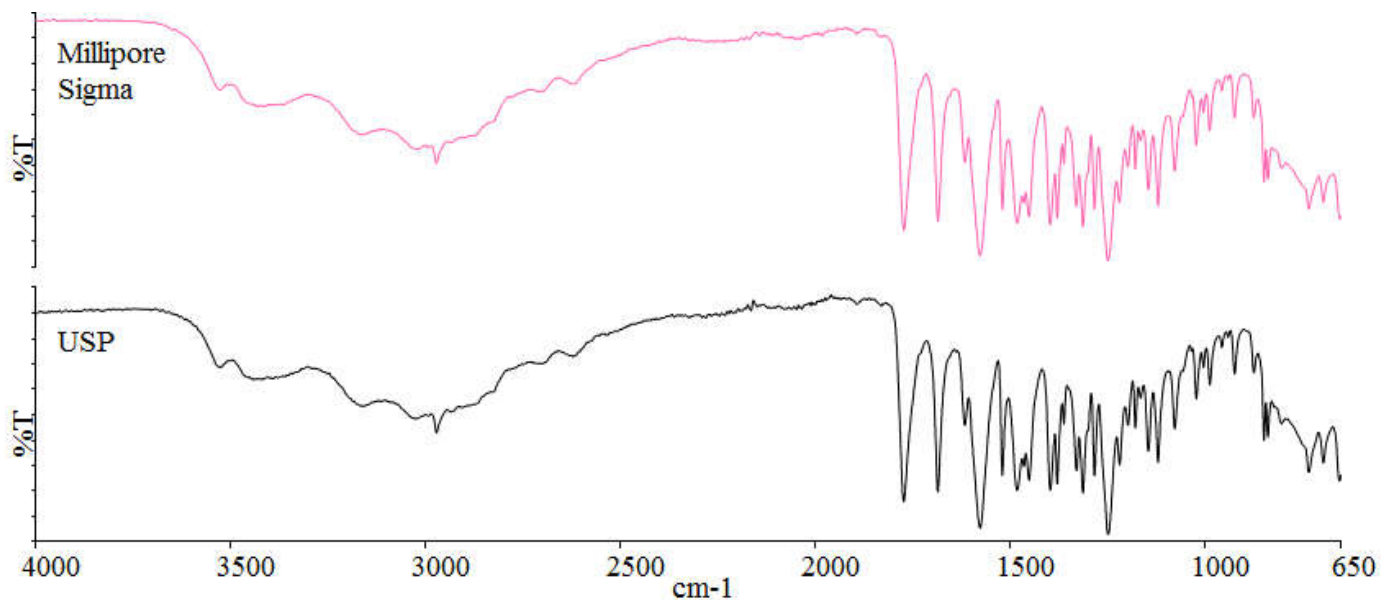
Sample size: ~60mg

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.

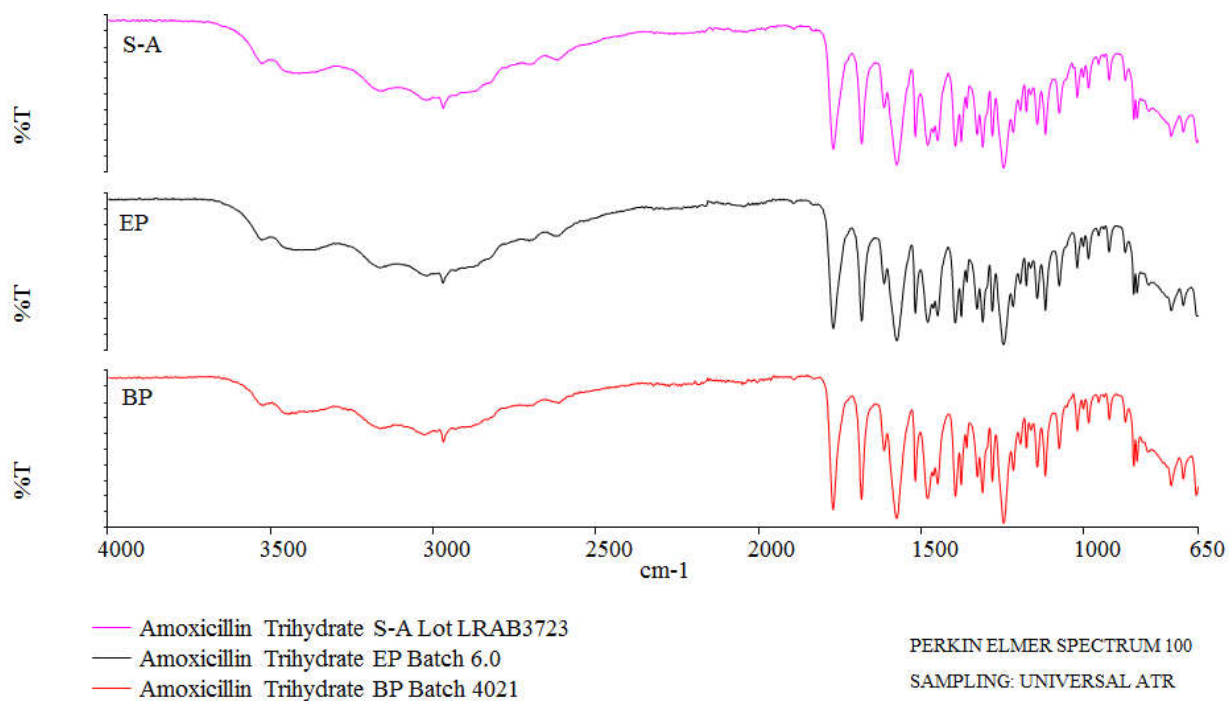
Long Term Stability Evaluation - An assessment, or re-test, versus a Compendial Reference Standard may be scheduled, within the 3 year anniversary date of a release of a Secondary Standard. The re-test interval will be determined on a case-by-case basis. Short Term Stability Study - It is useful to assess 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)



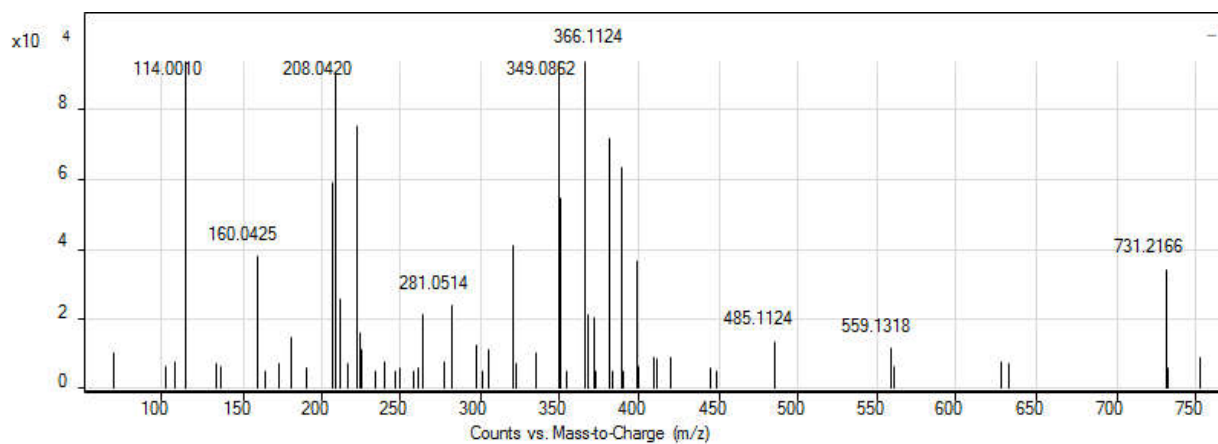
MilliporeSigma Lot: LRAB3723 vs. USP Lot R106H0



Indicative Values:

MASS SPECTRUM

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

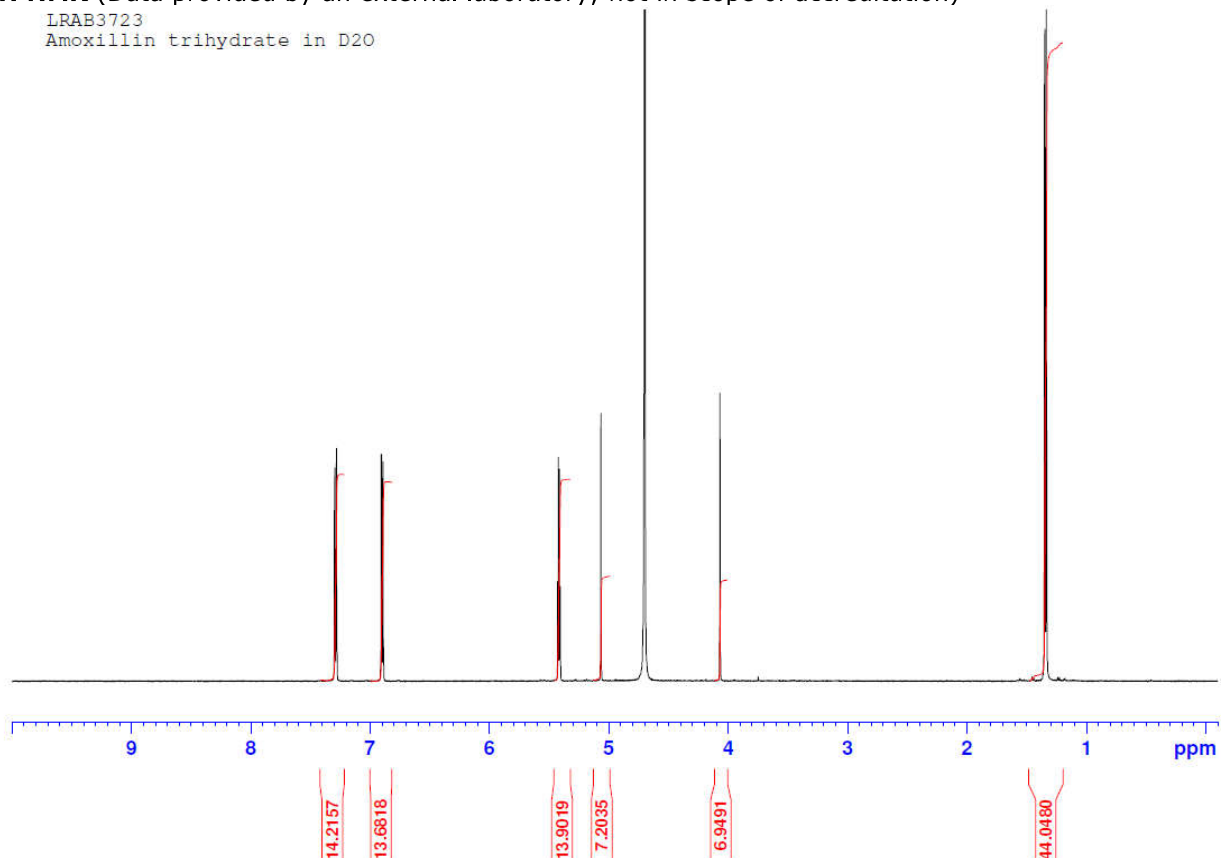


Theoretical value: 366.1124 m/z [M+H]⁺

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)

LRAB3723
Amoxicillin trihydrate in D2O



Consistent with structure

OPTICAL ROTATION

Specification: Specific Rotation +290° to +315° (EP)

Perkin Elmer Polarimeter 343

Wavelength: 589 nm

Concentration: 0.2g/100mL in water

Cell Path: 100 mm

Mean of three Measurements = + **304.19** °

Certificate of analysis revision history:

Certificate version	Date	Reason for version
LRAB3723.1	06 October 2017	Original Release
LRAB3723.2	31 August 2019	Stability & Requalification Test
LRAB3723.3	31 March 2020	Requalification Test

Disclaimer:

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