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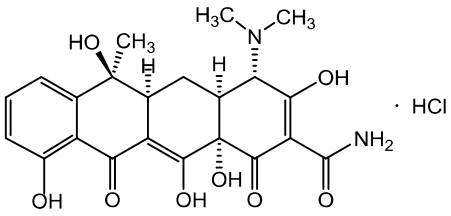
www.sigmaaldrich.com

Certificate of Analysis – Certified Reference Material

TETRACYCLINE HYDROCHLORIDE

Product no.:	PHR1041-500MG
Lot no.:	LRAC0159
Description of CRM:	Yellow Powder
Expiry date:	31 December 2023
Storage:	Store in Refrigerator/Protect from Light
Certificate version:	LRAC0159.3 (*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:	$C_{22}H_{25}ClN_2O_8$
Molecular mass:	480.90
CAS No.:	64-75-5



Analyte	Certified Purity ± associated uncertainty *U*, *U=k · u (k=)* (Mass Balance/ basis)
TETRACYCLINE HYDROCHLORIDE	**97.5 % Ucrm = ± 0.4 %, k = 2 (Mass Balance/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:	50 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:	13 January 2020

ANAB ACCREDITED ISO 17034 REFERENCE MATERIAL

ISO 17034
AR-1470

[Andy Ommen; Quality Control]

[Mark Pooler; Quality Assurance]

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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.

Packaging: 500 mg 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.

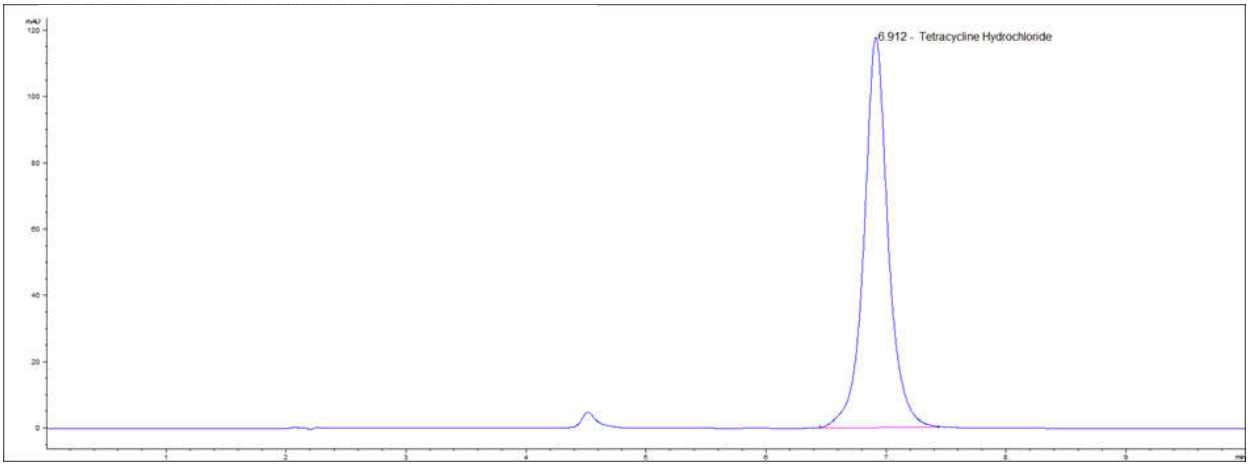
Traceability Assay:
Comparative assay demonstrates direct traceability to Pharmacopeial Standards

ASSAY vs. USP REFERENCE STANDARD (as is basis)

ASSAY VALUE	**vs. USP LOT**
95.1%	**R039W0** Labeled Content = 0.976 mg/mg

Method: HPLC (ref.: Tetracycline Hydrochloride, Current Compendial Monographs)
Column: Ascentis Express C8, 4.6 x 100 mm, 2.7 µm
Mobile Phase: 0.1 M Ammonium Oxalate: DMF: 0.2 M Dibasic Ammonium Phosphate (68:27:5), pH 7.7
Flow Rate: 0.4 mL/min
Column Temperature: 30 ºC
Injection: 2 µL
Detector: 280 nm

Representative Chromatogram from Lot: LRAC0159 vs USP Assay Analysis



ASSAY vs. EP CRS (as is basis)

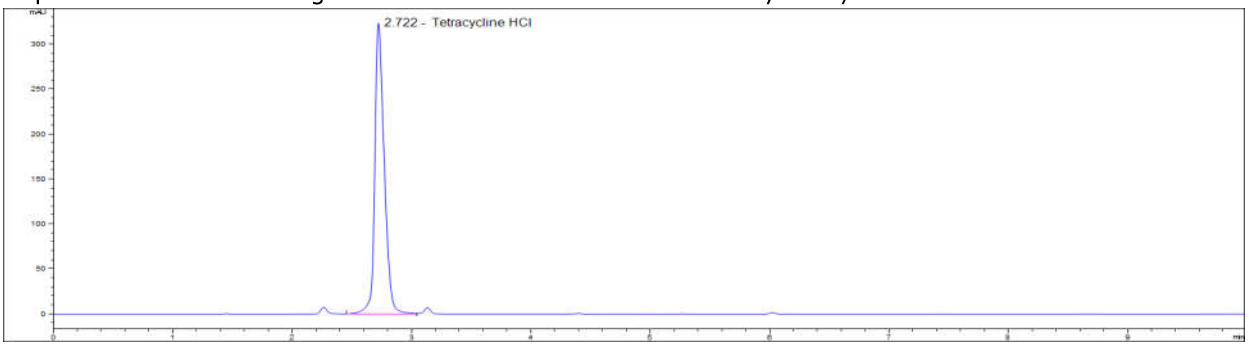
ASSAY VALUE	**vs. EP BATCH**
95.6%	**4.0** Labeled Content = None Assigned Content = 97.5 %

Method: HPLC (ref.: Tetracycline Hydrochloride, Current Compendial Monographs)
Column: Ascentis Express C18, 4.6 x 150 mm, 5 µm
Mobile Phase A: 0.1% H_3PO_4 in Water Mobile Phase B: Acetonitrile
Gradient:

Time (min)	% A	% B
0-7.5	85-60	15-40
7.5-7.6	60-85	40-15
7.6-10	85	15

Flow Rate: 1 mL/min
Column Temperature: 50 ºC
Injection: 5 µL
Detector: 280 nm

Representative Chromatogram from Lot: LRAC0159 vs EP Assay Analysis

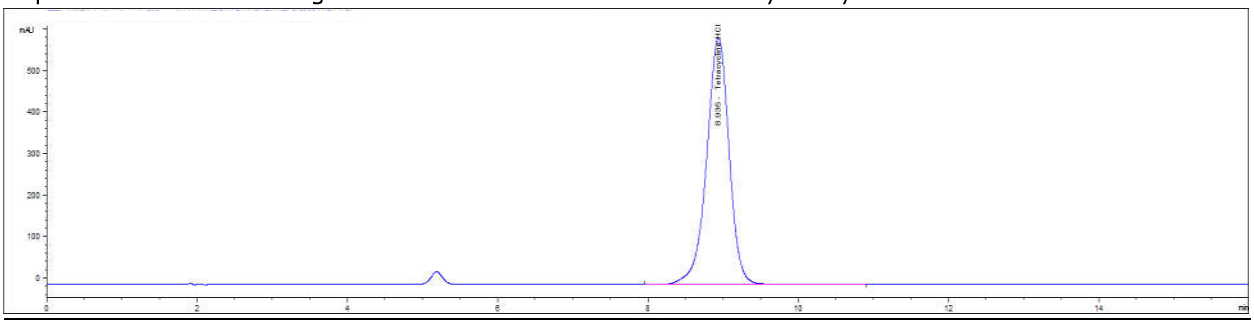


ASSAY vs. BP CRS (as is basis)

ASSAY VALUE	**vs. BP BATCH**
95.6%	**2833**
	Labeled Content = 96.1 %

Method: HPLC (ref.: Tetracycline Hydrochloride, Current Compendial Monographs)
Column: Wakosil II 5C8RS 4.6 x 250 mm, 5 µm
Mobile Phase: 0.1 M Ammonium Oxalate: DMF: 0.2 M Dibasic Ammonium Phosphate (68:27:5), pH 7.63
Flow Rate: 1.5 mL/min
Column Temperature: Ambient
Injection: 20 µL
Detector: 280 nm

Representative Chromatogram from Lot: LRAC0159 vs BP Assay Analysis



Certification process details:

The certified purity is determined by mass balance and calculated as

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

- TCI = Total Chromatographic Impurities
- LOD = Loss on Drying
- H_2O = 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:

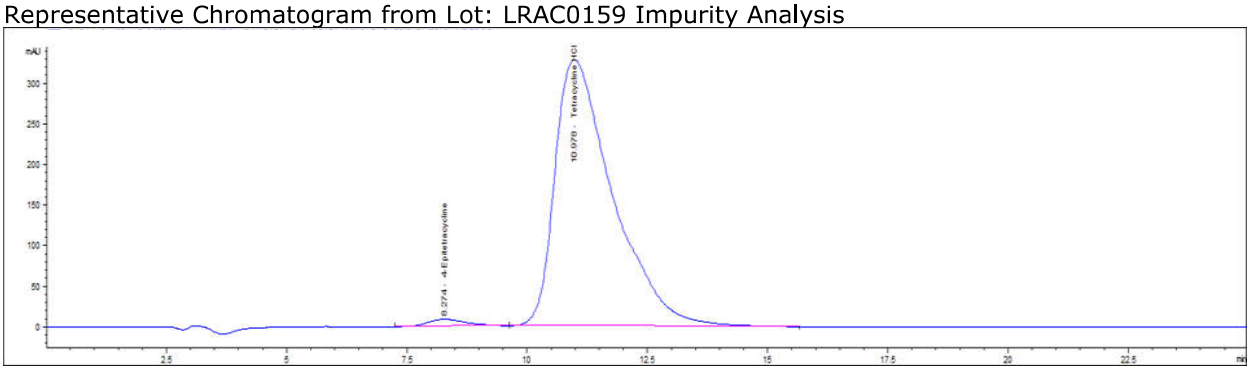
CHROMATOGRAPHIC IMPURITY ANALYSIS

METHOD: HPLC (ref.: Tetracycline Hydrochloride, Current Compendial Monographs)
Column: Hamilton PRP-1 4.6 x 250 mm, 5 µm
Mobile Phase: Tert-Butanol, Dipotassium Hydrogen Phosphate, Tetrabutylammonium Sulphate, Sodium Edetate/Water; pH 9.0
Flow Rate: 1 mL/min
Column Temperature: 60 °C
Injection: 20 µl
Detector: 254 nm

Impurities Detected:

Impurity A (4-epitetracycline) = **1.9%**

Representative Chromatogram from Lot: LRAC0159 Impurity Analysis



RESIDUAL SOLVENTS

Method: GC-MS Headspace (ref.: Adapted from Residual Solvents USP <467>)
Column: DB-1301
Carrier gas: He
Flow: 1.2 mL/min
Split Ratio: 1:5
Injection/Temperature: 1 µL/250 °C
Temperature Program: 40 °C for 20 min, 10 °C/min to 240 °C, hold 20 min

Solvents Detected: **None**

LOSS ON DRYING/VOLATILES

Method: Oven at 105 ºC (ref.: Current Compendial Monographs)
Mean of three measurements, Loss = **0.4%**

RESIDUE ANALYSIS

Method: Sulfated Ash (ref.: Current Compendial Monographs)
Sample Size: ~ 1 g
Mean of three measurements, Residue = **0.2%**

CERTIFIED PURITY BY MASS BALANCE

97.5 % U_{crm} = ± 0.4 %, k = 2
(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.

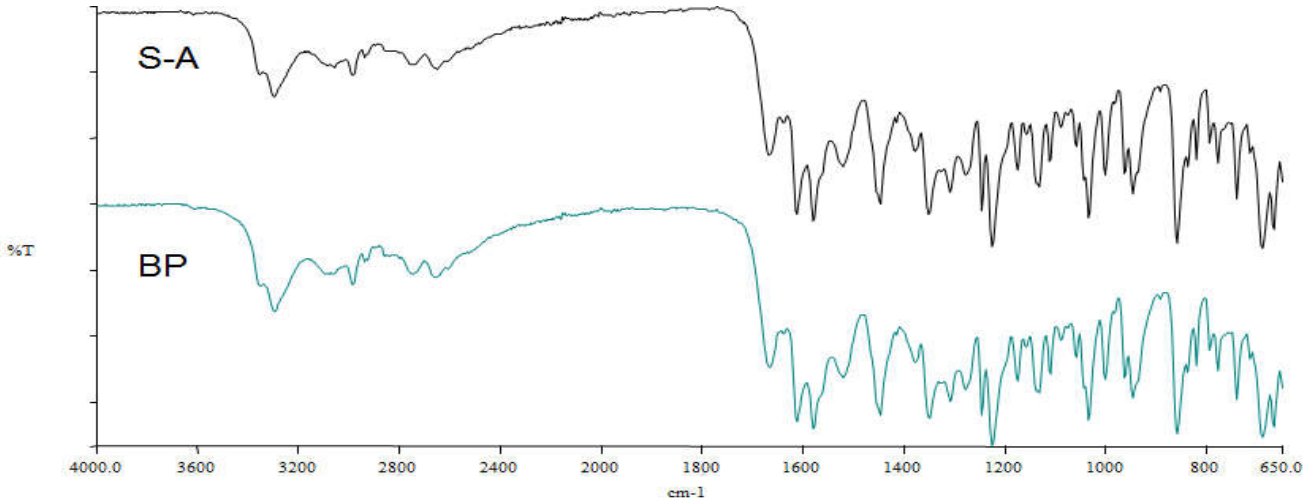
Analytical method: HPLC Sample size: ~ 50 mg

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.

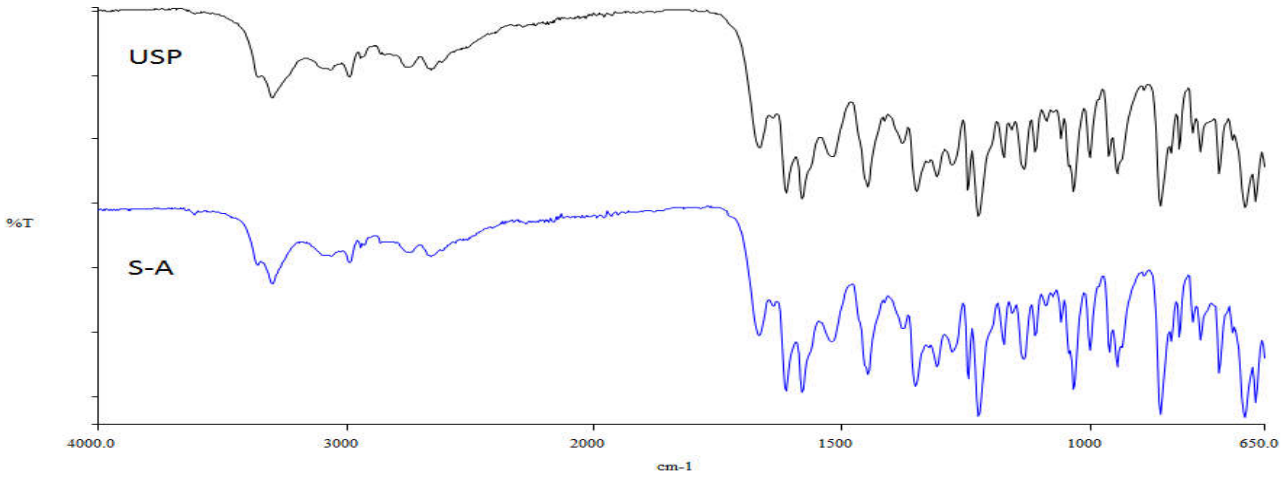
Long Term Stability Evaluation - An assessment, or re-test, versus a Compendial Reference Standard may be scheduled, within the 3 year anniversary date of a release of a Secondary Standard. The re-test interval will be determined on a case-by-case basis. Short Term Stability Study - It is useful to assess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

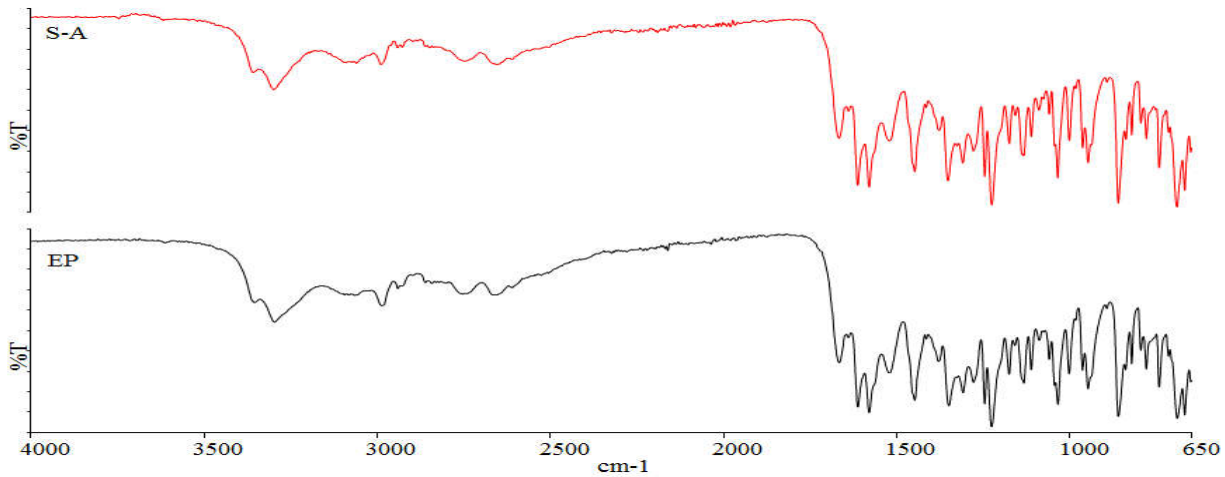
INFRARED SPECTROPHOTOMETRY (Comparative identification analysis demonstrates direct traceability to Pharmacopeial standards)



S-A Lot LRAC0159 vs BP Batch 2833



USP Lot R039W0 vs S-A Lot LRAC0159

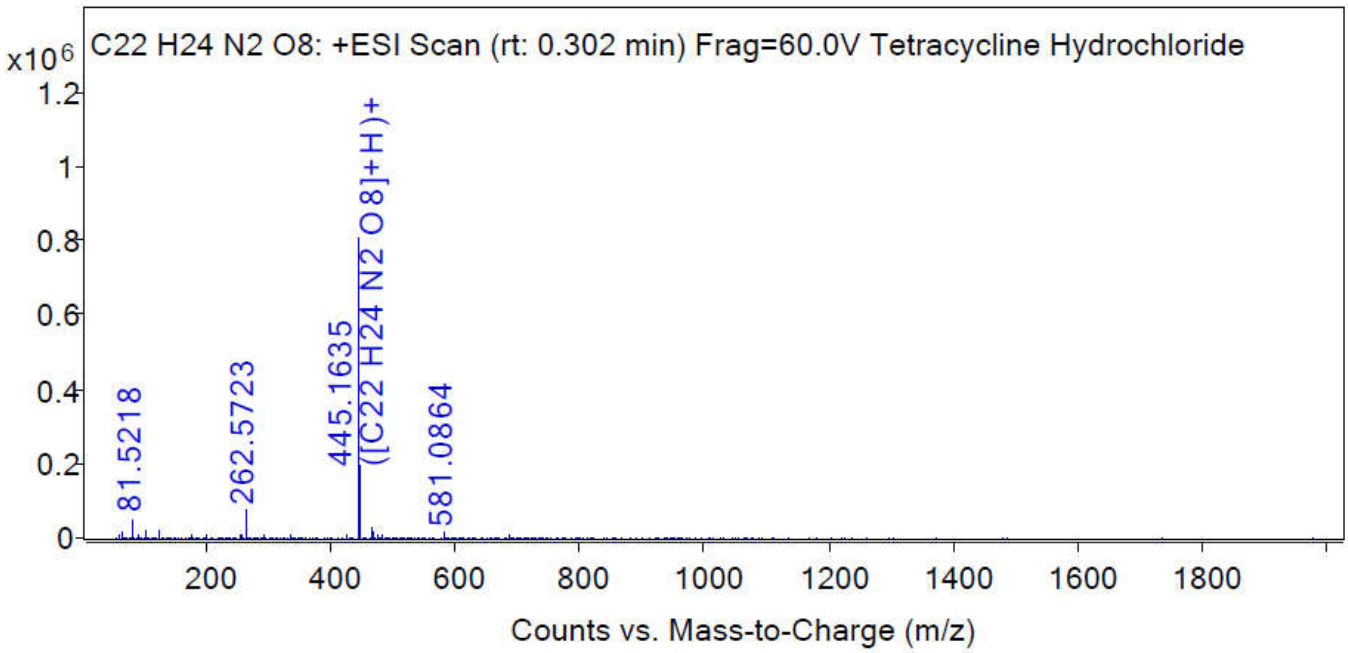


S-A Lot LRAC0159 vs EP Batch 4.0

Indicative Values:

MASS SPECTRUM

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

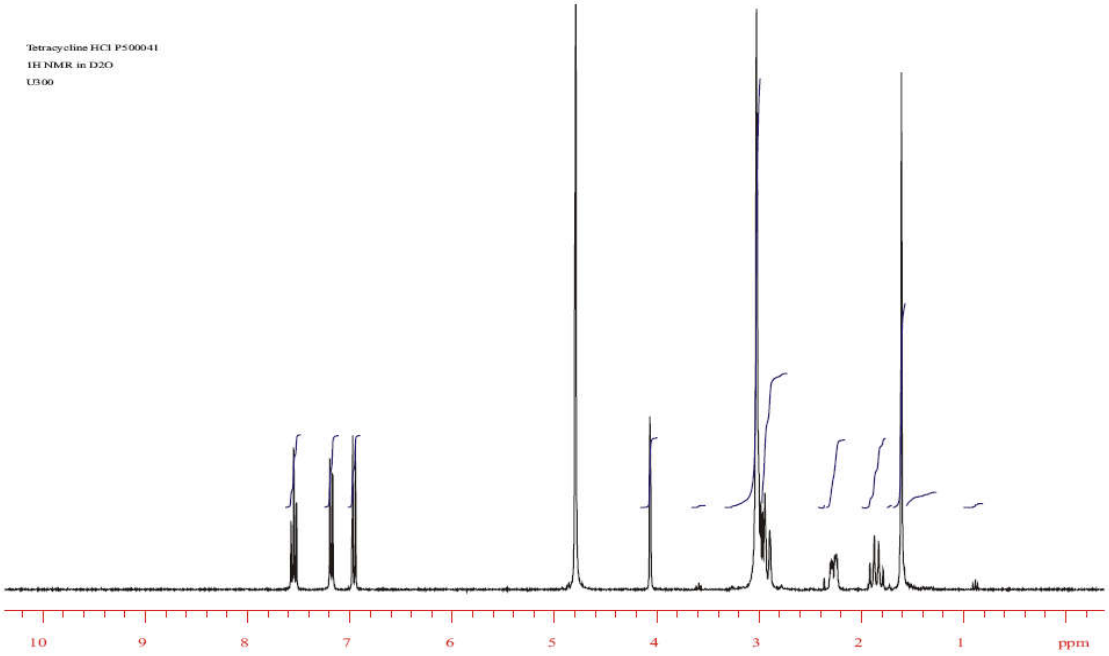


Theoretical value: 445.1611 m/z

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

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

LRAC0159



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	54.95	54.80	54.78	54.79
H	5.24	5.22	5.25	5.24
N	5.83	5.92	5.85	5.89

OPTICAL ROTATION
Perkin Elmer Polarimeter 343
Wavelength: 589nm
Concentration: ~ 1 g/100 mL
Cell Path: 100 mm
Mean of three Measurements = **-241.1°**

Certificate of analysis revision history:

Certificate version	Date	Reason for version
LRAC0159.1	17 August 2018	Original Release
LRAC0159.2	31 October 2019	Stability & Requalification Test
LRAC0159.3	13 January 2020	CoA Content Update

Disclaimer:

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