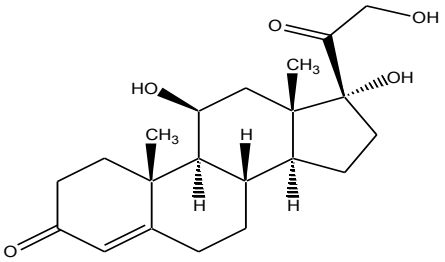
Certificate of Analysis

ISO GUIDE 34
ACLASS Cert# AR-1470

ISO/IEC 17025
ACLASS Cert# AT-1467

HYDROCORTISONE

CERTIFIED REFERENCE MATERIAL



CERTIFIED PURITY:

98.7%, U_{crm} = ±0.7% k = 2 (Mass Balance/dried basis)

98.8%, U_{crm} = ±0.7% k = 2 (Mass Balance/as is basis)

NOMINAL PACKAGE SIZE: 500mg

CATALOG #: PHR1014 **LOT #:** LRAA5629

CERTIFICATE VERSION: LRAA5629.1 **ISSUE DATE:** 07 July 2014

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.*

CRM EXPIRATION: 31 December 2019 (Proper Storage and Handling Required).

RECEIPT DATE: _____

Note: this space is provided for convenience only and its use is not required.

STORAGE: Store at Room Temperature, keep container tightly closed. Attachment of a 20 mm aluminum crimp seal recommended for unused portions.

CHEMICAL FORMULA: $C_{21}H_{30}O_5$ **MW:** 362.47

PHYSICAL DESCRIPTION: White powder in amber vial **CAS #:** 50-23-7

HAZARDS: Read Safety Data Sheet before using. All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel.

INSTRUCTIONS FOR USE: For USP applications, dry at 105°C for 3 hours. For EP and BP applications, 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. This material is intended for R&D use only. Not for drug, household or other uses.

TRACEABILITY ASSAY

Comparative assay demonstrates direct traceability to Pharmacopeial Standards
Specification: 97.0 to 102.0% (USP)

METHOD: HPLC (ref.: USP 36, Hydrocortisone)
Column: Exsil ODS, 4.6 x 250mm, 5µm
Mobile Phase: Water, Acetonitrile, Methanol (50:25:25)
Flow Rate: 1mL/min
Column Temperature: Ambient
Injection: 10µl
Detector: 254nm
Internal Standard: Propylparaben

ASSAY vs. USP REFERENCE STANDARD (dried basis)

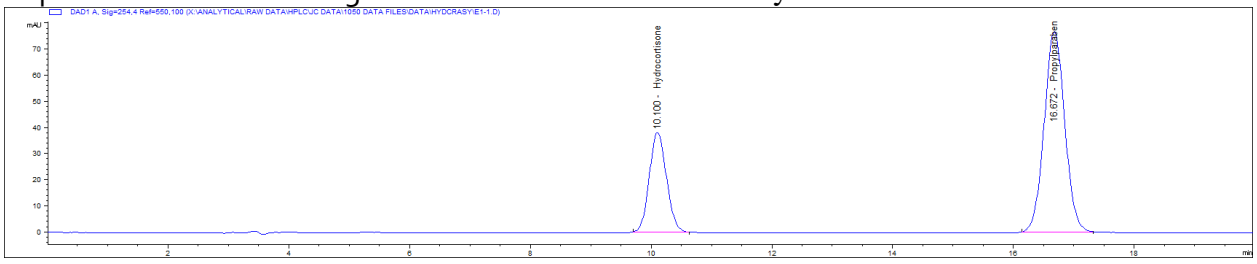
ASSAY VALUE	vs. USP LOT
99.4%	**N0F289**
	Labeled Content = 0.997 mg/mg

ASSAY vs. EP CRS (as is basis)

ASSAY VALUE	vs. EP BATCH
98.8%	**8**
	Labeled Content = None
	Assigned Content = 99.0%*

*The assigned content of the EP CRS was determined by assay against the BP CRS

Representative Chromatogram from Lot: LRAA5629 Analysis

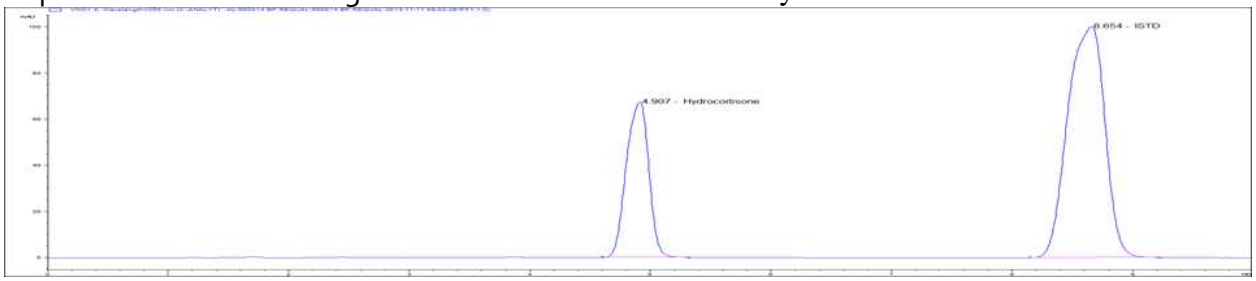


Column: Ascentis C18, 4.6 x 250mm, 5µm
Mobile Phase: Water, Acetonitrile, Methanol (50:25:25)
Flow Rate: 1mL/min
Column Temperature: 30ºC
Injection: 10µl
Detector: 254nm
Internal Standard: Propylparaben

ASSAY vs. BP CRS (as is basis)

ASSAY VALUE	vs. BP BATCH
98.3%	**3497**
	Labeled Content = 99.8%

Representative Chromatogram from Lot: LRAA5629 Analysis



PURITY DETERMINATION BY MASS BALANCE

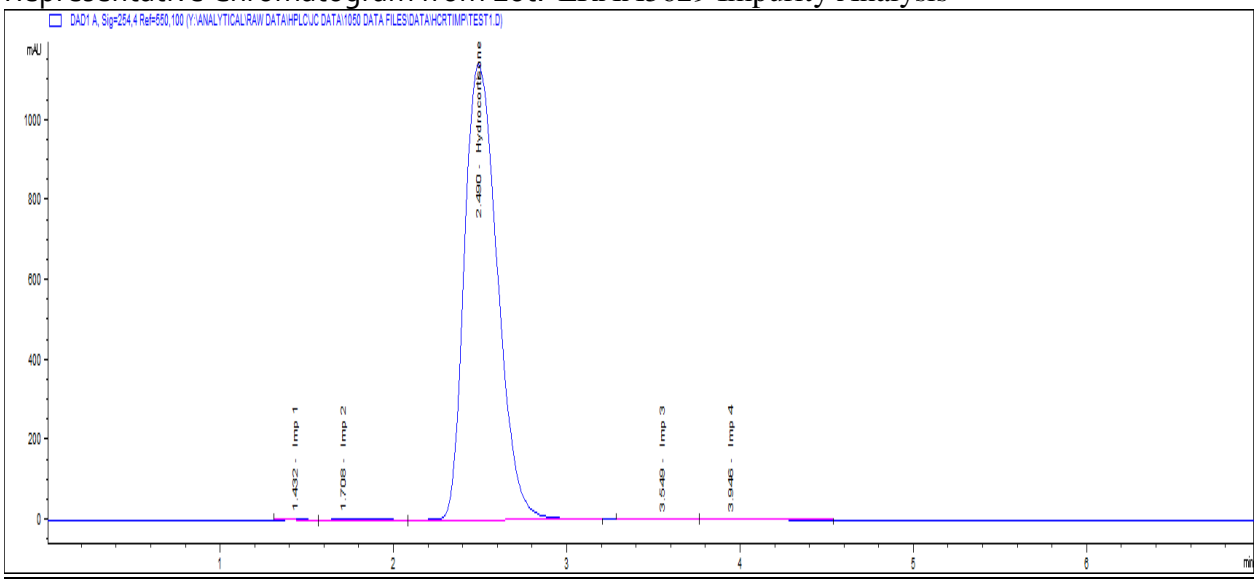
CHROMATOGRAPHIC IMPURITY ANALYSIS
METHOD: HPLC (ref.: USP 31, Hydrocortisone)
Column: Exsil Silica, 4.6 x 250mm, 5µm
Mobile Phase: Butyl chloride, THF, Methanol, glacial acetic acid, Water (890:56:28:24:0.04)
Flow Rate: 1.5mL/min
Column Temperature: Ambient
Injection: 10µl
Detector: 254nm

Impurities Detected:

Impurity 1:	**0.3%**
Impurity 2:	**0.5%**
Impurity 3:	**0.3%**
Impurity 4:	**0.07%**

Total Impurities: **1.2%**

Representative Chromatogram from Lot: LRAA5629 Impurity Analysis



RESIDUAL SOLVENTS

Method: GC-MS Headspace (ref.: Residual Solvents <467>, USP34)
Column: DB-1301
Carrier gas: He
Flow: 1.2mL/min
Split Ratio: 1:5
Injection/Temperature: 1µl/250°C
Temperature Program: 40°C for 20min, 10°C/min to 240°C, hold 20min

Solvents Detected: None

LOSS ON DRYING/VOLATILES

Method: Oven at 105°C
Mean of three measurements, Loss = **0.06%**

RESIDUE ANALYSIS

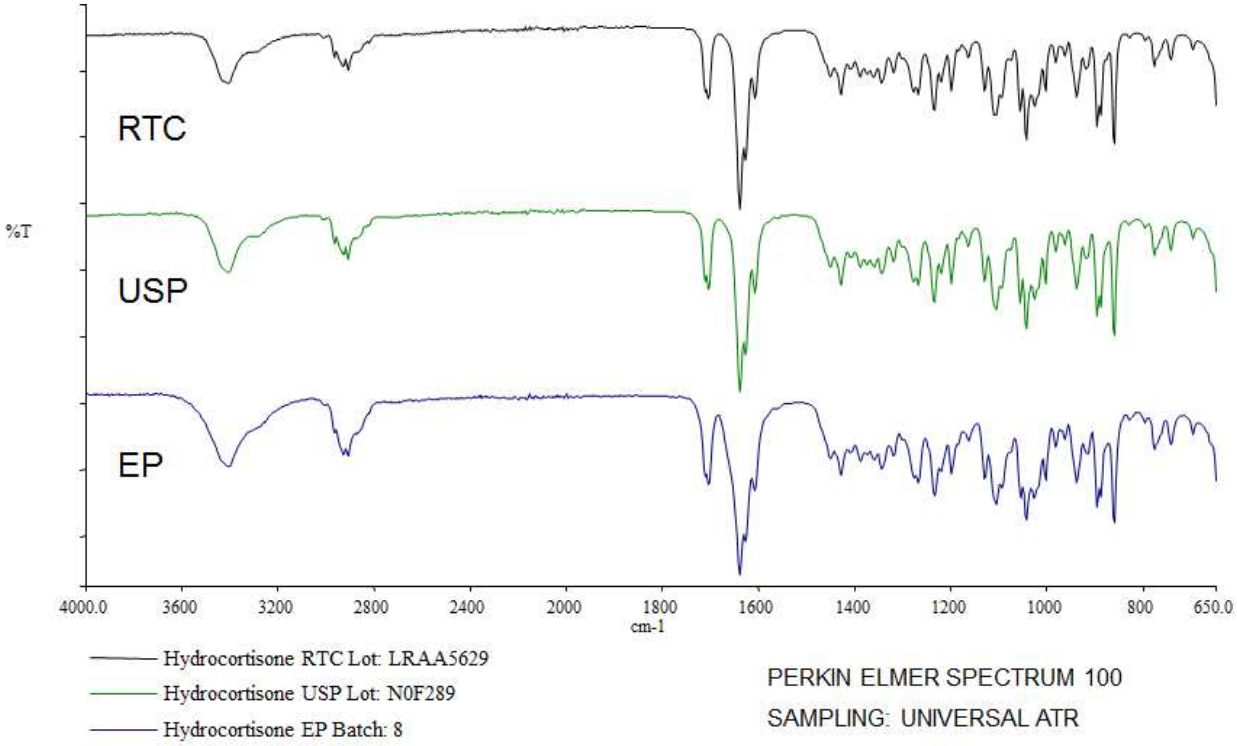
Method: Sulfated Ash
Sample Size: ~100 mg
Mean of three measurements, Residue = **0%**

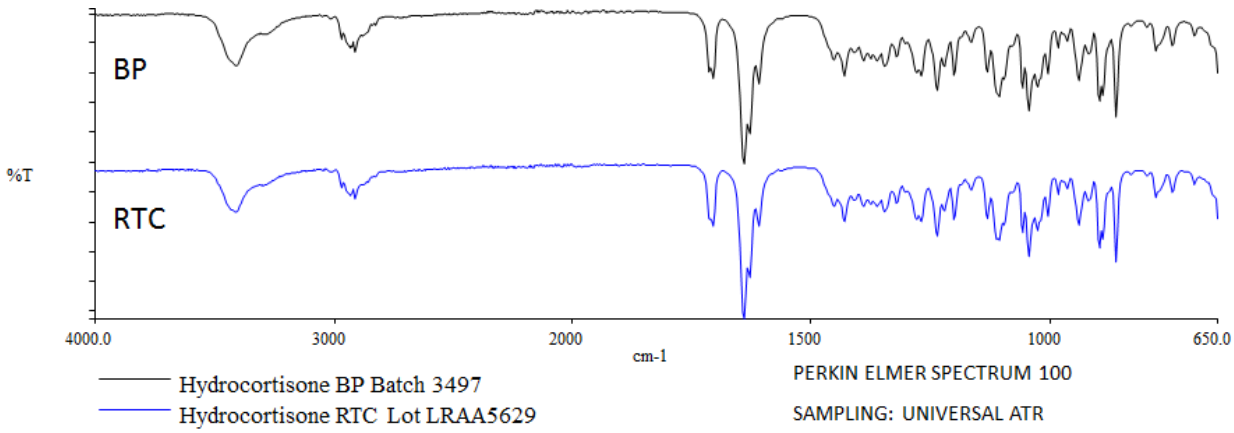
CERTIFIED PURITY BY MASS BALANCE [100% - Impurities (normalized)]

98.7% U_{crm} = ±0.7%, k = 2 (dried basis)
98.8% U_{crm} = ±0.7%, k = 2 (as is basis)

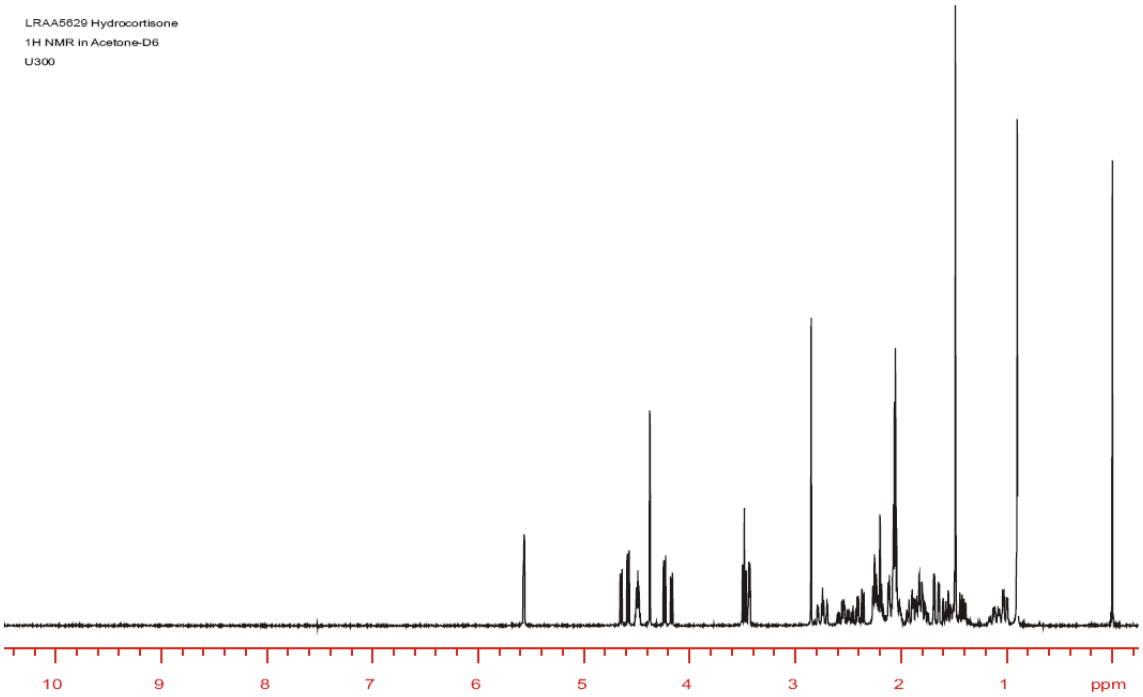
IDENTIFICATION TESTS

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





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



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	69.59	69.49	69.51	69.50
H	8.34	8.36	8.40	8.38

OPTICAL ROTATION

Specification: Between +150° and +156° (USP/EP)
Perkin Elmer Polarimeter 343
Wavelength: 589nm
Concentration: 10 mg/mL in Dioxane
Cell Path: 100mm
Mean of three Measurements = **+153.6** °

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

UNCERTAINTY STATEMENT

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.

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.

Operations Manager

QA Supervisor

APPENDIX

Original Release Date: 07 July 2014

Manufactured and certified by Sigma-Aldrich RTC, Inc.
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ACCREDITED REFERENCE MATERIAL PRODUCER

ACCREDITED TESTING