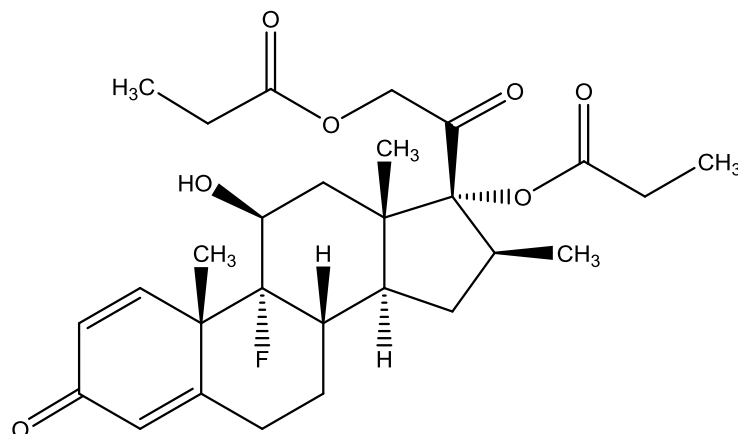


# Certificate of Analysis

**ISO GUIDE 34**  
ANAB Cert# AR-1470

**ISO/IEC 17025**  
ANAB Cert# AT-1467

## BETAMETHASONE DIPROPIONATE CERTIFIED REFERENCE MATERIAL



### CERTIFIED PURITY:

**99.6%**,  $U_{\text{crm}} = \pm 0.1\%$   $k = 2$  (Mass Balance/dried basis)

**99.4%**,  $U_{\text{crm}} = \pm 0.2\%$   $k = 3.18$  (Mass Balance/as is basis)

**NOMINAL PACKAGE SIZE:** 1g

**CATALOG #:** PHR1399

**LOT #:** LRAA9185

**CERTIFICATE VERSION:** LRAA9185.2

**ISSUE DATE:** 31 October 2015

*Note: Certificates may be updated due to Pharmacopeial Lot changes or the availability of new data.*

*Check our website at: [www.sigma-aldrich.com](http://www.sigma-aldrich.com) for the most current version.*

**CRM EXPIRATION:** 31 December 2020 (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:**  $\text{C}_{28}\text{H}_{37}\text{FO}_7$

**MW:** 504.6

**PHYSICAL DESCRIPTION:** White powder in amber vial

**CAS #:** 5593-20-4

**SIGMA-ALDRICH®**

**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 portion at 105°C for 3 hours prior to use. For EP applications, 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. 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: 97.0-103.0% (USP)

### ***ASSAY vs. USP REFERENCE STANDARD (dried basis)***

#### **ASSAY VALUE**

**100.5%**

#### **vs. USP LOT**

**R02220**

Labeled Content = 0.990mg/mg

#### **METHOD: HPLC (ref.: Betamethasone Dipropionate, USP38)**

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

Mobile Phase: Acetonitrile, Water (50:50)

Flow Rate: 1mL/min

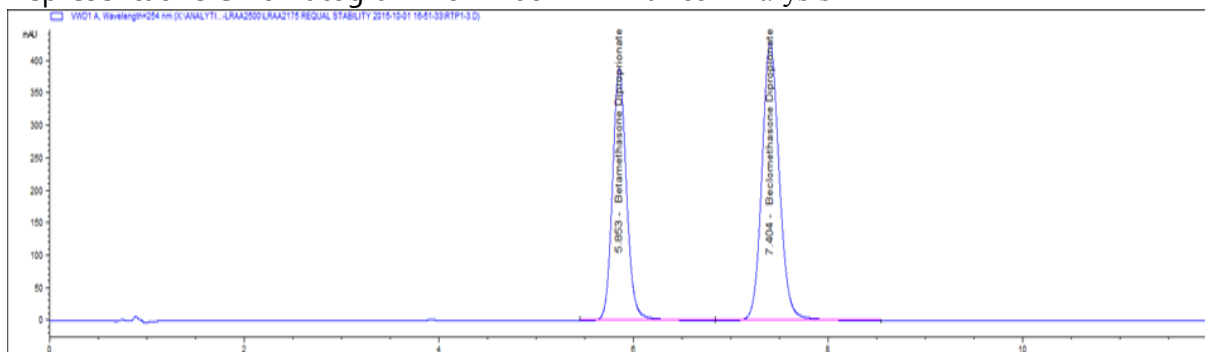
Column Temperature: 30°C

Injection: 10µL

Detector: 254nm

Internal Standard: Beclomethasone Dipropionate

#### **Representative Chromatogram from Lot: LRAA9185 Analysis**



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

### ASSAY VALUE

**100.0%**

### vs. EP BATCH

**3.0**

Labeled Content = 99.5%

Column: Ascentis Express C18, 4.6 x 100mm, 5 $\mu$ m

Mobile Phase: Acetonitrile, Water (50:50)

Flow Rate: 1mL/min

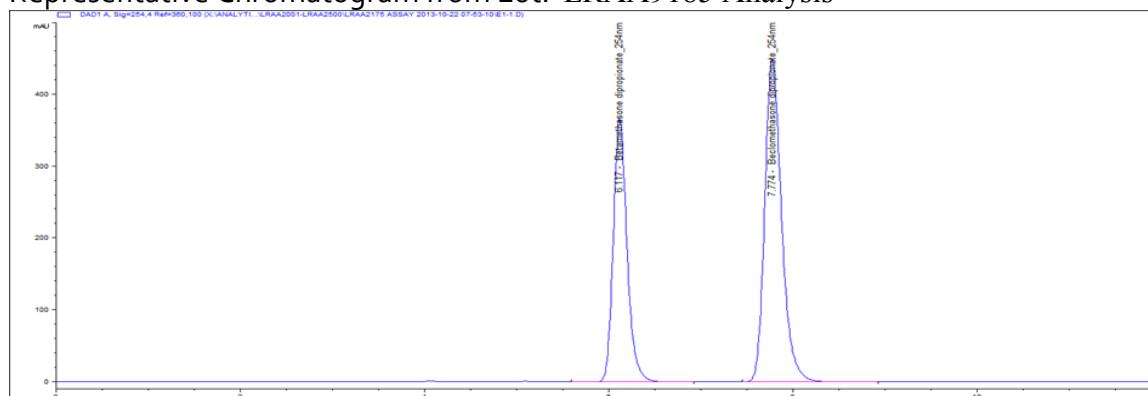
Column Temperature: 30°C

Injection: 10 $\mu$ L

Detector: 254nm

Internal Standard: Beclomethasone Dipropionate

### Representative Chromatogram from Lot: LRAA9185 Analysis



## PURITY DETERMINATION BY MASS BALANCE

### CHROMATOGRAPHIC IMPURITY ANALYSIS

**METHOD: HPLC (ref.: Betamethasone Dipropionate, USP36)**

Column: Ascentis Express C18, 4.6 x 100mm, 5 $\mu$ m

Mobile Phase: Acetonitrile, Water (55:45)

Flow Rate: 1mL/min

Column Temperature: 30°C

Injection: 10 $\mu$ L

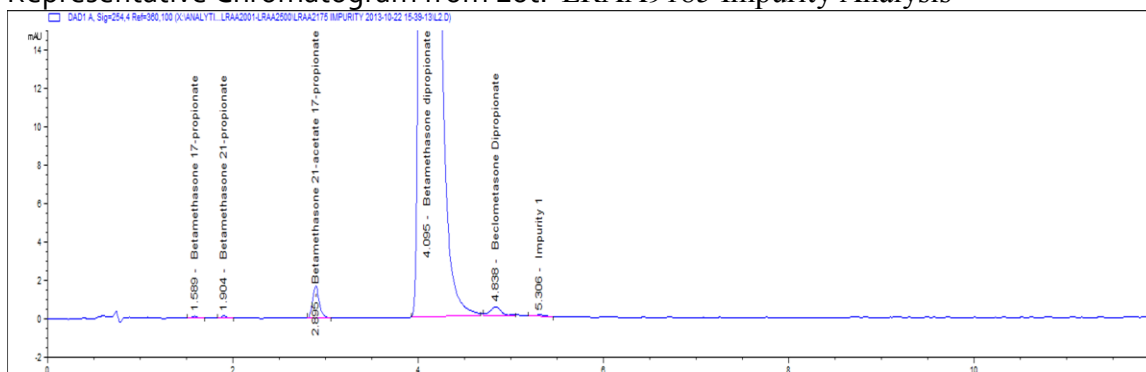
Detector: 254nm

#### Impurities Detected:

Betamethasone 17-propionate:	0.01%
Betamethasone 21-propionate:	0.01%
Betamethasone 21-acetate 17-propionate:	0.1%
Beclometasone Dipropionate:	0.04%
Impurity 1:	0.01%

Total Impurities: **0.2%**

#### Representative Chromatogram from Lot: LRAA9185 Impurity Analysis



#### RESIDUAL SOLVENTS

Method: GC-MS Headspace (ref.: Adapted from Residual Solvents <467>, USP34)

Column: SPB-624

Carrier gas: He

Flow: 10.2mL/min

Split Ratio: 1:5

Injection/Temperature: 1µl/250°C

Temperature Program: 40°C for 5min, 8°C/min to 200°C, hold 5min

#### Solvents Detected:

Diisopropyl Ether: **0.1%**

#### LOSS ON DRYING/VOLATILES

Method: Oven at 105°C

Mean of three measurements, Loss = **0.2%**

#### RESIDUE ANALYSIS

Method: Sulfated Ash

Sample Size: ~1g

Mean of three measurements, Residue = **0.01%**

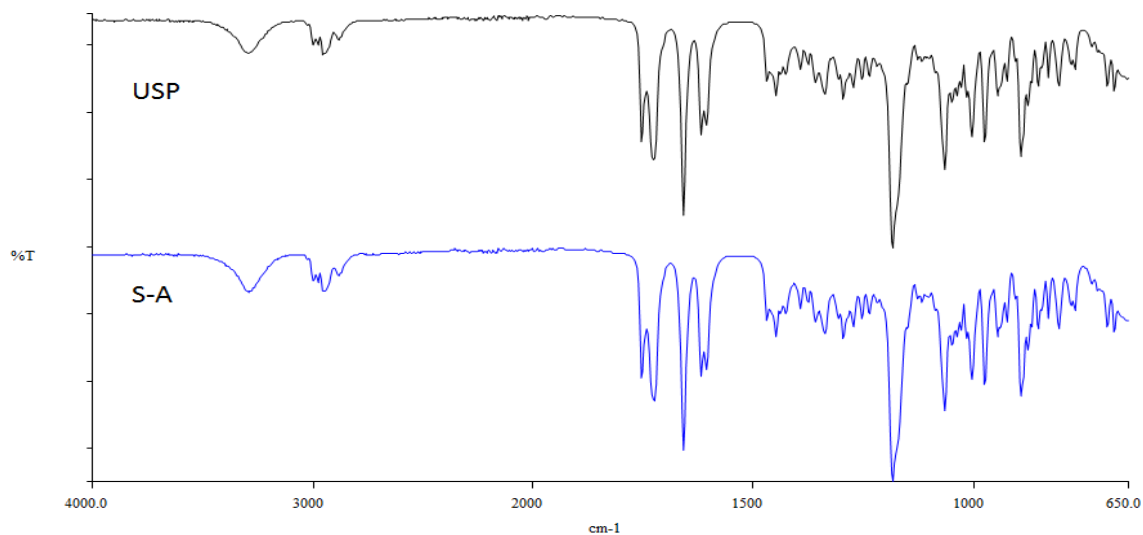
## **CERTIFIED PURITY BY MASS BALANCE** [100% - Impurities (normalized)]

**99.6%**  $U_{\text{crm}} = \pm 0.1\%$ ,  $k = 2$  (dried basis)

**99.4%**  $U_{\text{crm}} = \pm 0.2\%$ ,  $k = 3.18$  (as is basis)

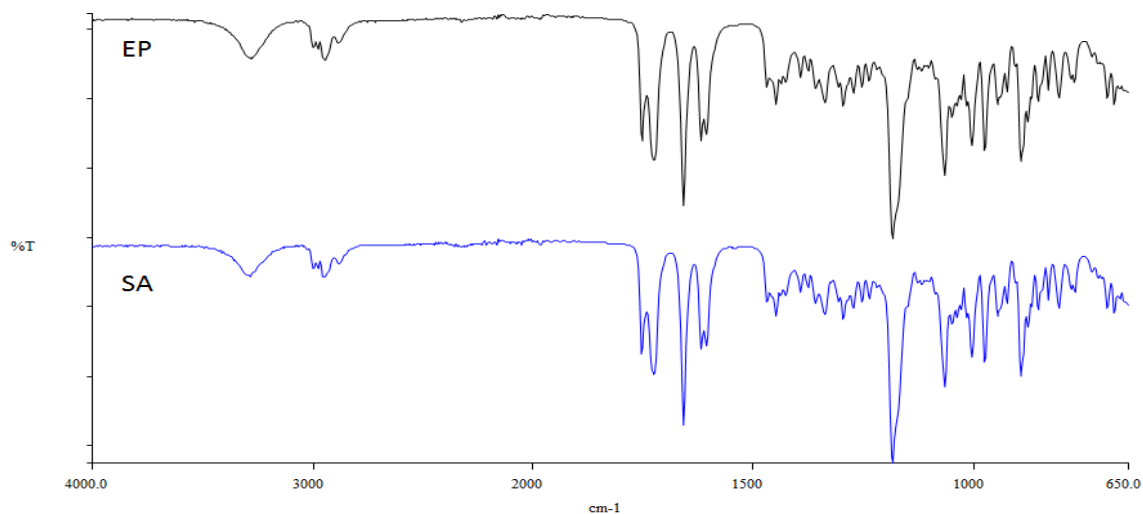
## **IDENTIFICATION TESTS**

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



— Betamethasone Dipropionate USP Lot R02220  
— Betamethasone Dipropionate S-A Lot LRAA9185

PERKIN ELMER SPECTRUM 100  
SAMPLING: UNIVERSAL ATR

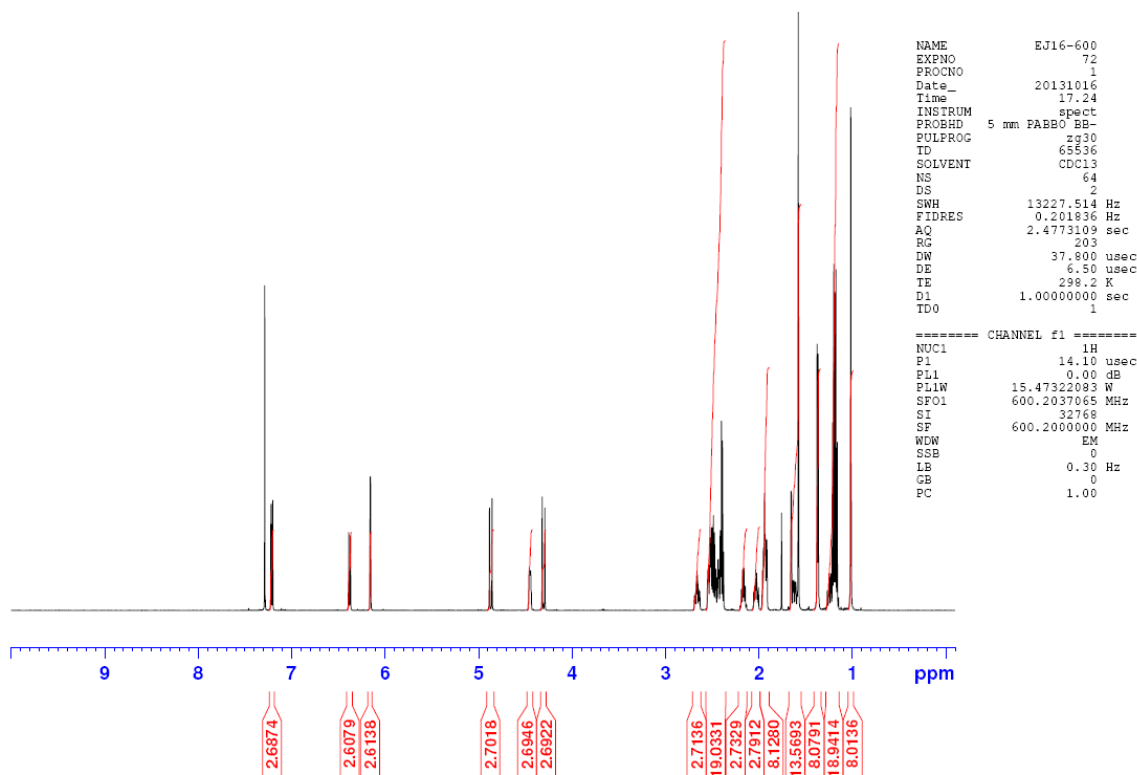


— Betamethasone Dipropionate EP Batch 3.0  
— Betamethasone Dipropionate SA Lot LRAA9185

PERKIN ELMER SPECTRUM 100  
SAMPLING: UNIVERSAL ATR

# <sup>1</sup>H NMR (Data provided by an external laboratory; not in scope of accreditation)

LRAA9185 Betamethasone Dipropionate in CDC13



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	66.65	66.78	66.56	66.67
H	7.39	7.52	7.25	7.39

## OPTICAL ROTATION

Specification: +63° to +70°

Perkin Elmer Polarimeter 343

Wavelength: 589nm

Concentration: ~1g/100mL

Cell Path: 100mm

Mean of three Measurements = +67.1°

### 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: ~30mg

### UNCERTAINTY STATEMENT

Uncertainty values in this document are expressed as Expanded Uncertainty ( $U_{\text{crm}}$ ) corresponding to the 95% confidence interval.  $U_{\text{crm}}$  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.



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QC Supervisor



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QA Supervisor

### APPENDIX

Original Release Date: 30 September 2015  
Stability Test Date: 31 October 2015  
Requalification Test Date: 31 October 2015