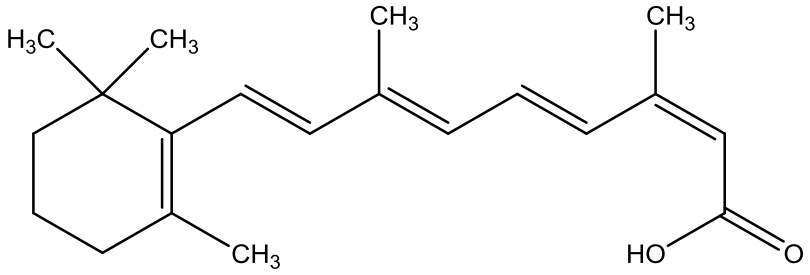
Certificate of Analysis

ISO GUIDE 34
ANAB Cert# AR-1470

ISO/IEC 17025
ANAB Cert# AT-1467

ISOTRETINOIN

CERTIFIED REFERENCE MATERIAL



CERTIFIED PURITY: 98.5%, U_{crm} = ±1.0% k = 2.13
(Mass Balance/as is basis)

NOMINAL PACKAGE SIZE: 3x100mg

CATALOG #: PHR1188 **LOT #:** P500188

CERTIFICATE VERSION: 500188.3 **ISSUE DATE:** 31 May 2016

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: 12 Months from Receipt (Proper Storage and Handling Required).

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

STORAGE: Store in a Freezer/Protect from Light.

CHEMICAL FORMULA: $C_{20}H_{28}O_2$ **MW:** 300.4

PHYSICAL DESCRIPTION: Orange powder in amber ampule **CAS #:** 4759-48-2

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: Do not dry, use on the as is basis. Allow ampule to reach room temperature before opening. Use the content of the vial promptly. 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

ASSAY vs. USP REFERENCE STANDARD (as is basis)

ASSAY VALUE	vs. USP LOT
99.3%	**R017X0**
	Labeled Content = 0.997mg/mg

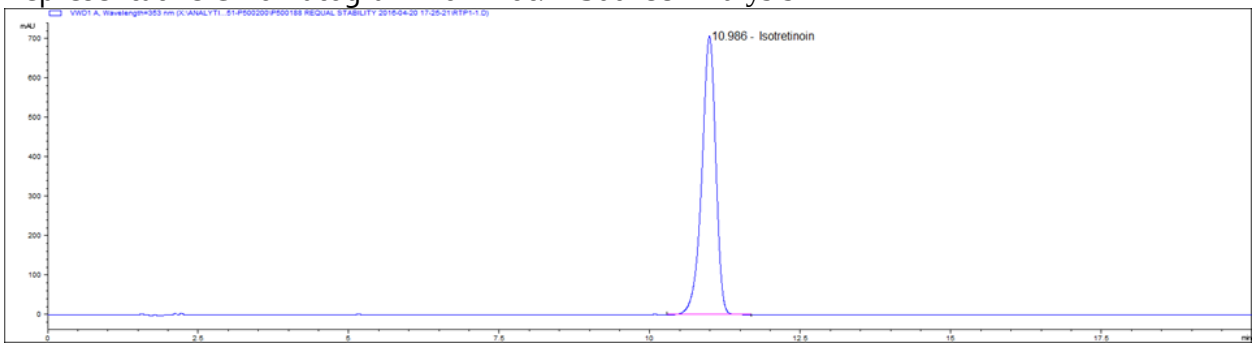
ASSAY vs. EP CRS (as is basis)

ASSAY VALUE	vs. EP BATCH
99.3%	**5.0**
	Labeled Content = None
	Assigned Content = 76.6%*

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

METHOD: HPLC (ref.: Isotretinoin Capsules, USP38)
Column: Ascentis C18, 250 x 4.6mm, 5µm
Mobile Phase: 0.5% Acetic acid in Methanol, 0.5% Acetic acid in Water (88:12)
Flow Rate: 1.5mL/min
Column Temperature: 30ºC
Injection: 20µL
Detector: 353nm

Representative Chromatogram from Lot: P500188 Analysis



PURITY DETERMINATION BY MASS BALANCE

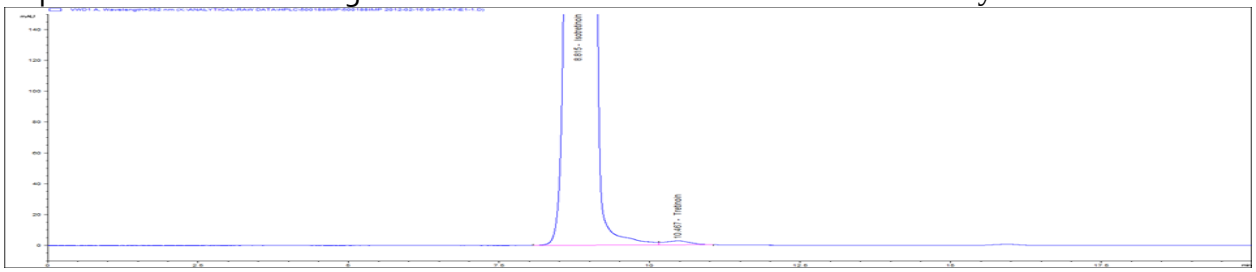
CHROMATOGRAPHIC IMPURITY ANALYSIS

LIMIT OF TRETINOIN

METHOD: HPLC (ref.: Isotretinoin, USP34)
Column: Ascentis Si, 250 x 4.6mm, 5µm
Mobile Phase: Isooctane, Isopropyl alcohol, Acetic acid (99.65:0.25:0.1)
Flow Rate: 1.0mL/min
Column Temperature: 30ºC
Injection: 20µL
Detector: 352nm

Total Tretinoin: **0.3%**

Representative Chromatogram from Lot: P500188 Limit of Tretinoin Analysis



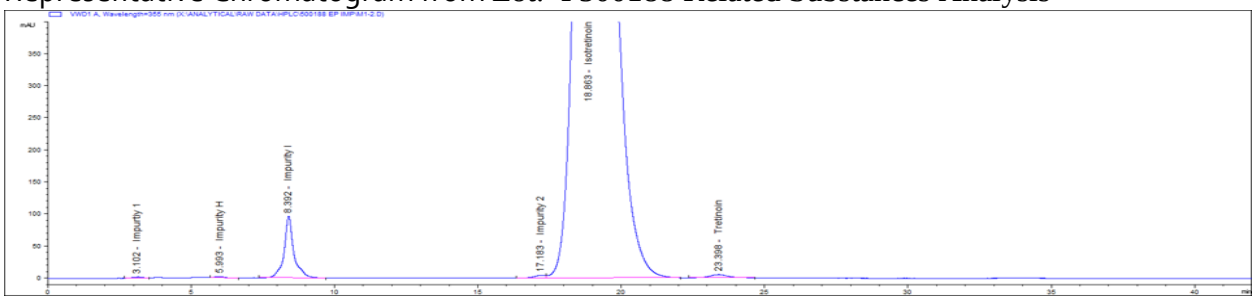
RELATED SUBSTANCES

METHOD: HPLC (ref.: Isotretinoin, EP7)
Column: Ascentis C18, 250 x 4.6mm, 5µm
Mobile Phase: Solution A, Solution B (70:30)
Solution A: Methanol, Water, Acetic Acid (770:225:5)
Solution B: Methanol, Acetic Acid (995:5)
Flow Rate: 1mL/min
Column Temperature: 30ºC
Injection: 10µL
Detector: 355nm
Impurities Detected:

Impurity 1: **0.03%**
Impurity H: **0.02%**
Impurity I: **1.0%**
Impurity 2: **0.07%**

Total Impurities: **1.1%**

Representative Chromatogram from Lot: P500188 Related Substances 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

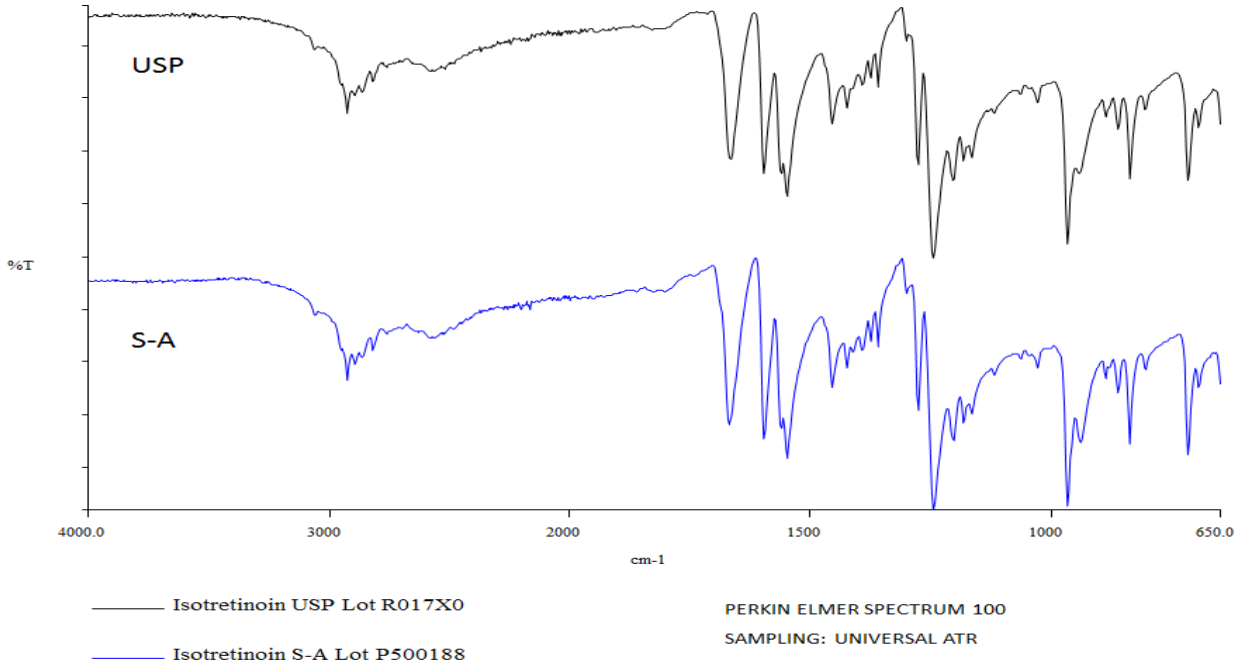
Method: Sulfated Ash
Sample Size: ~1g
Mean of three measurements, Residue = **0.03%**

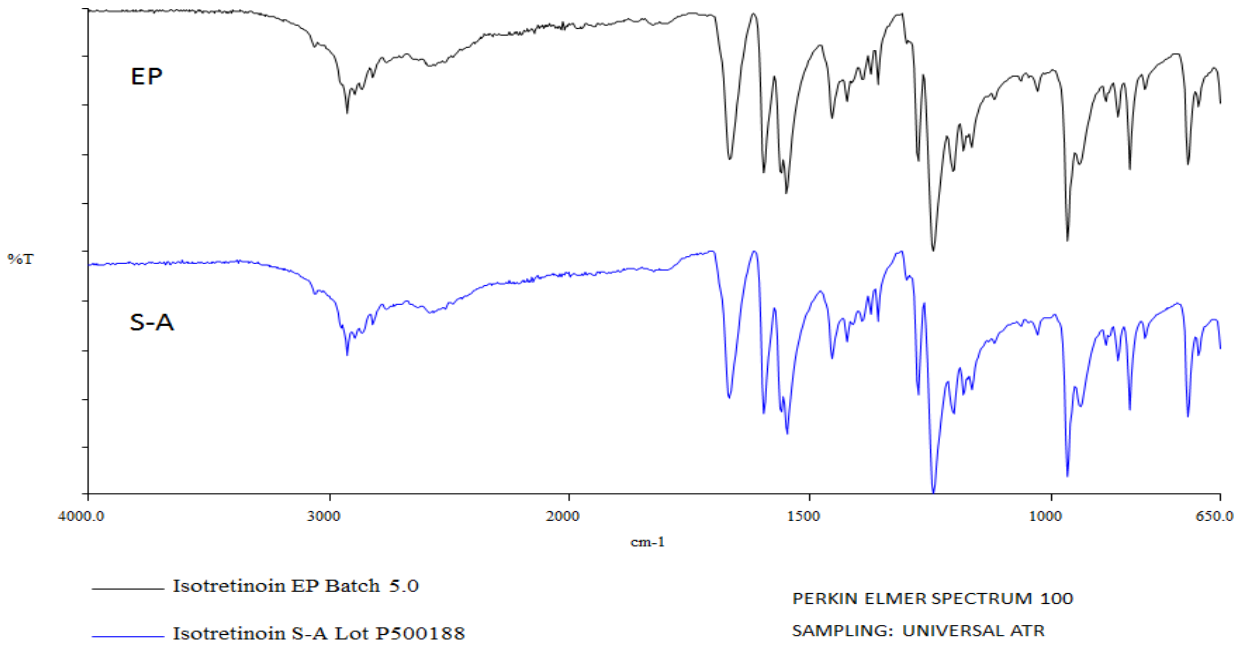
CERTIFIED PURITY BY MASS BALANCE [100% - Impurities (normalized)]

98.5% U_{crm} = ±1.0%, k = 2.13
(as is basis)

IDENTIFICATION TESTS

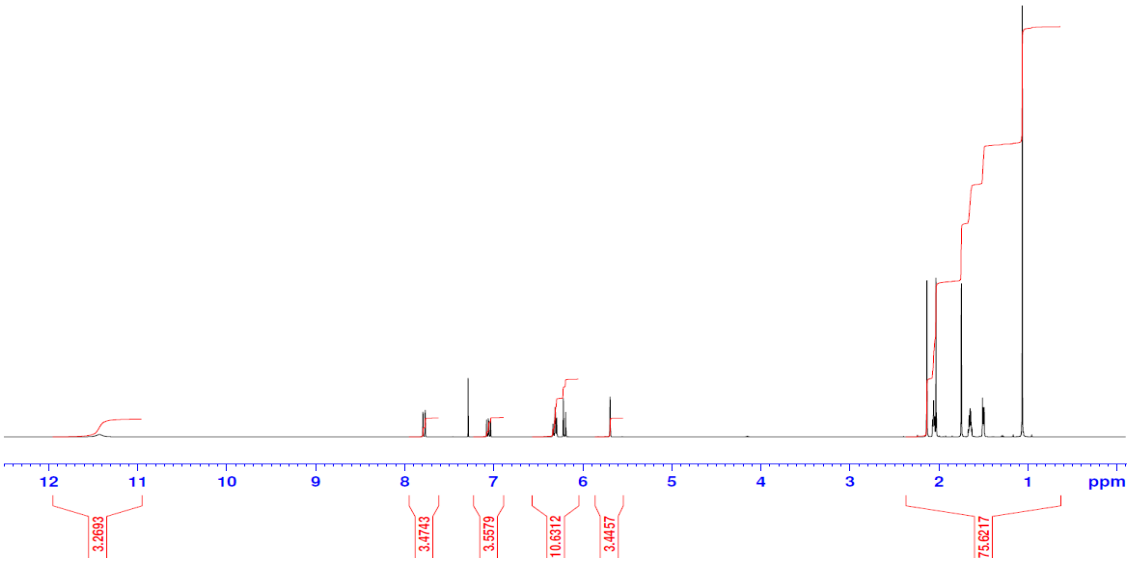
INFRARED SPECTROPHOTOMETRY (Comparative identification analysis demonstrates direct traceability to Pharmacopeial standards)





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

P500188 Istotretinoin in CDCl3



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	79.95	80.08	80.04	80.06
H	9.39	9.56	9.52	9.54

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: ~50mg

UNCERTAINTY STATEMENT

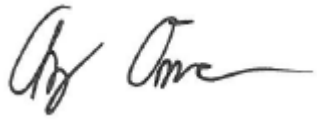
Uncertainty values in this document are expressed as Expanded Uncertainty (U_{crm}) corresponding to the 95% confidence interval. U_{crm} is derived from the combined standard uncertainty multiplied by the coverage factor k, which is obtained from a *t*-distribution and degrees of freedom. The components 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.



QC Supervisor

QA Supervisor

APPENDIX

Original Release Date:	04 May 2012
Stability Test Date:	22 April 2015
Requalification Test Date:	29 May 2015
Requalification Test Date:	31 May 2016

Manufactured and certified by Sigma-Aldrich RTC, Inc.
2931 Soldier Springs Rd, Laramie WY, USA 82070
(Phone): 1-307-742-5452 (Fax): 1-855-831-9212
email: RTCTechGroup@sial.com