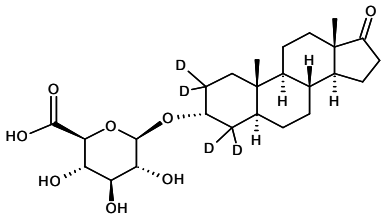
Cerilliant
Analytical Reference Standards
a SIGMA-ALDRICH company

Cerilliant Quality
ISO GUIDE 34
ISO/IEC 17025
ISO 13485
ISO 9001
GMP/GLP

Certificate of Analysis

Androsterone-D_4 glucuronide



Catalog Number: A-099
Solution Lot: FE011312-01
Retest Date: February 2015
Solvent: Methanol
Volume per Ampule: Not less than 1 mL
Storage: Store unopened in freezer.
Shipping: Ship cold. See Stability Section.
Intended Use: For R&D/ analytical purposes only. Not suitable for human or animal consumption.
Regulatory: Canadian TK# 61-671 **Safety:** Flammable, Poison

- Retest Date - stability studies ongoing. Certificate of Analysis will be updated upon completion of retest.
- Ampules are overfilled to ensure a minimum 1 mL volume fill. We advise laboratories to use measured volumes of this standard solution before diluting to the desired concentration.

Component	Solution Purity	Certified Concentration
Androsterone-D_4 glucuronide	99.6%	100.0 ± 0.9 µg/mL

- Uncertainty of the concentration is expressed as an expanded uncertainty in accordance with ISO 17025 and Guide 34 at the approximate 95% confidence interval using a coverage factor of k = 2 and has been calculated by statistical analysis of our production system and incorporates uncertainty of the purity factor, material density, and balance and weighing technique.
- This standard is prepared gravimetrically and mass results are reported on the conventional basis for weighing in air. Concentration is calculated based on: the actual measured mass; Purity Factor of the analyte(s); measured mass of the solution; and the density of the pure diluent at 20°C.
- Concentration is corrected for chromatographic purity, residual water, residual solvents and residual inorganics.

Solution Standard Verification and Homogeneity

Standard Solution	Lot Number	Verified Concentration (µg/mL)		%RSD - Homogeneity	
		Actual Results	Acceptance Crtieria	Actual Results	Acceptance Crtieria
New Lot	FE011312-01	99.7	± 5%	1.1	≤ 3%

- Concentration is verified through multiple analyses and is calculated as the average of multiple analyses compared to an independently prepared calibration solution.
- Homogeneity of the New Lot is ensured through rigorous production process controls statistically analyzed to evaluate risk and verified by analysis. The % RSD of samples pulled from across the lot demonstrate homogeneity of the New Lot.

Traceability

- Gravimetrically prepared using qualified balances calibrated semi-annually by Mettler Toledo using NIST traceable weights. Calibration verification performed weekly and prior to each use utilizing NIST traceable weights. Each balance has been assigned a minimum weighing by Mettler Toledo taking into consideration the balance and installed environmental conditions to ensure weighing complies with USP tolerances of no more than 0.1% relative error.
- Concentration is verified against an independently prepared calibration solution gravimetrically prepared using balances calibrated to NIST.
- In addition, each neat material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques. Spectral data is provided on subsequent pages of the COA.

Cerilliant certifies that this standard meets the specifications stated in this certificate and warrants this product to meet the stated acceptance criteria through the expiration/retest date when stored unopened as recommended. Product should be used shortly after opening to avoid concentration changes due to evaporation. Warranty does not apply to ampoules stored after opening.

ACLASS ACCREDITED REFERENCE MATERIAL PRODUCER

Darron Ellsworth, Quality Assurance Manager

April 15, 2014
Date

Standard Solution Assay Parameters

Analysis Method: LC/MS
Column: Luna 3µ C18, 2.0 x 100 mm
Mobile Phase: Acetonitrile::0.1% Formic acid (40::60)
Flow Rate: 0.3 mL/min
Ionization: Electrospray, Negative Ion

Calibration Curve

Calibration Curve: Linear Regression
Number of Points: 3
Linearity (r): 0.990

Neat Material Data

Compound Name: Androsterone-D_4 glucuronide
Compound Lot: FN120911-02

Chemical Formula: $C_{23}H_{34}D_4O_8$
CAS Number: NA
Molecular Weight: 470.59

Neat Material Characterization Summary

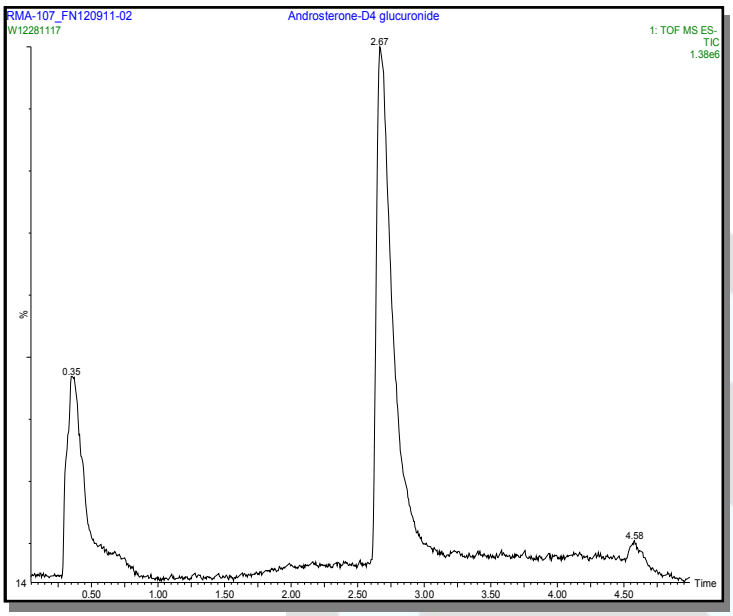
Analytical Test	Method	Results	
Chromatographic Purity by LC/MS Analysis	SP10-0107	> 99.9%	
Identity by LC/MS Analysis	SP10-0107	Consistent with Structure	
Isotopic Purity by LC/MS SIM Analysis	SP10-0107	0.03% D_0 vs D_4	
		0.02% D_0	21.92% D_3
		0.16% D_1	75.28% D_4
		2.62% D_2	
Identity by ^{1}H-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure	
Residual Solvent Analysis by GC/FID Headspace	AM1087 [1]	1.42%	
Residual Water Analysis by Karl Fischer Coulometry	USP <921>, SP10-0103	0.44%	
Inorganic Content by Microash Analysis	SP10-0135	< 0.2%	
Assay by Quantitative NMR Analysis	USP <761>, SP10-0116	90.33%	
Purity Factor		90.33%	

- Chromatographic purity is calculated as the average of two independently performed analyses utilizing two different methods. Acceptance criteria requires the purity values to be within 0.5% of each other.

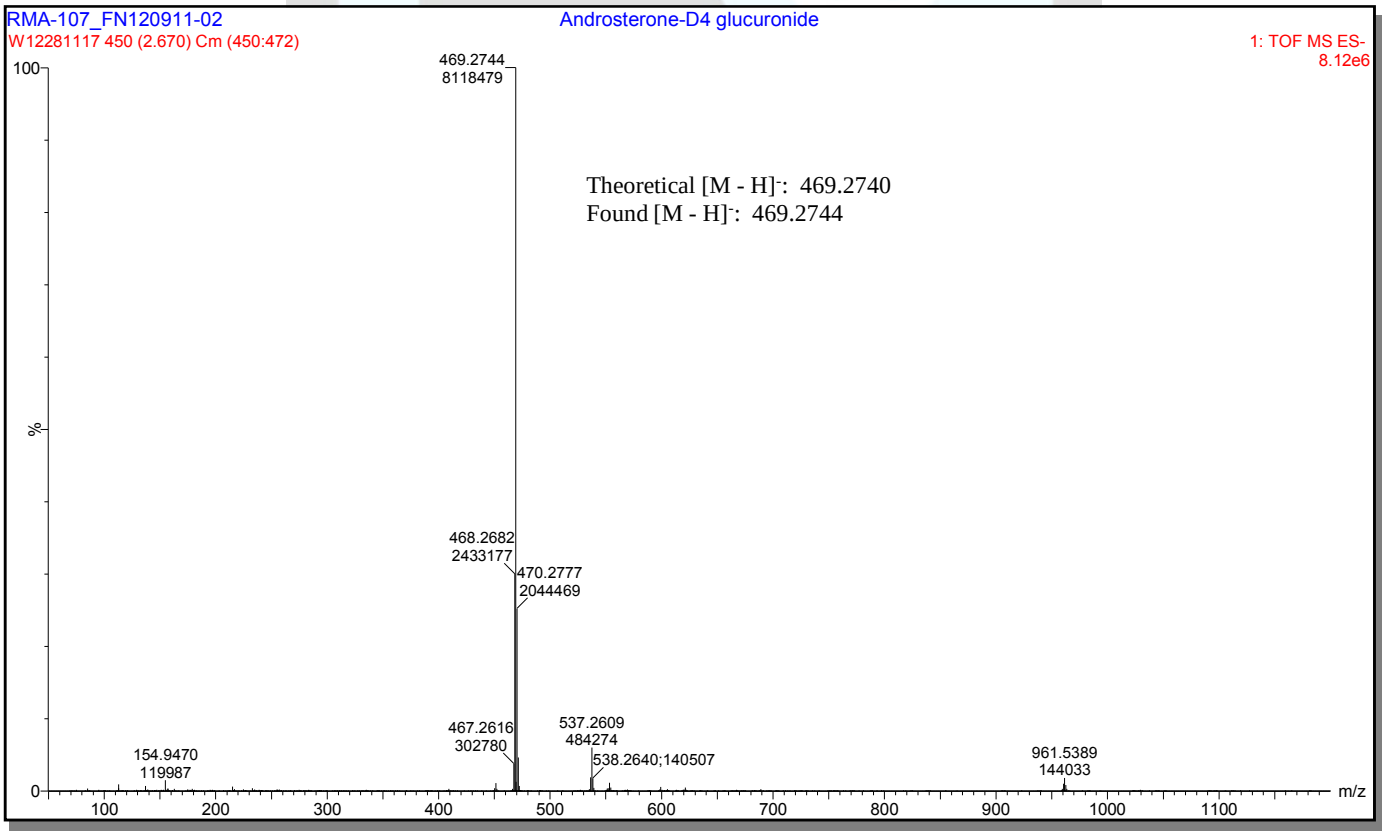
[1] Validated analytical method

Spectral and Physical Data

LC/MS



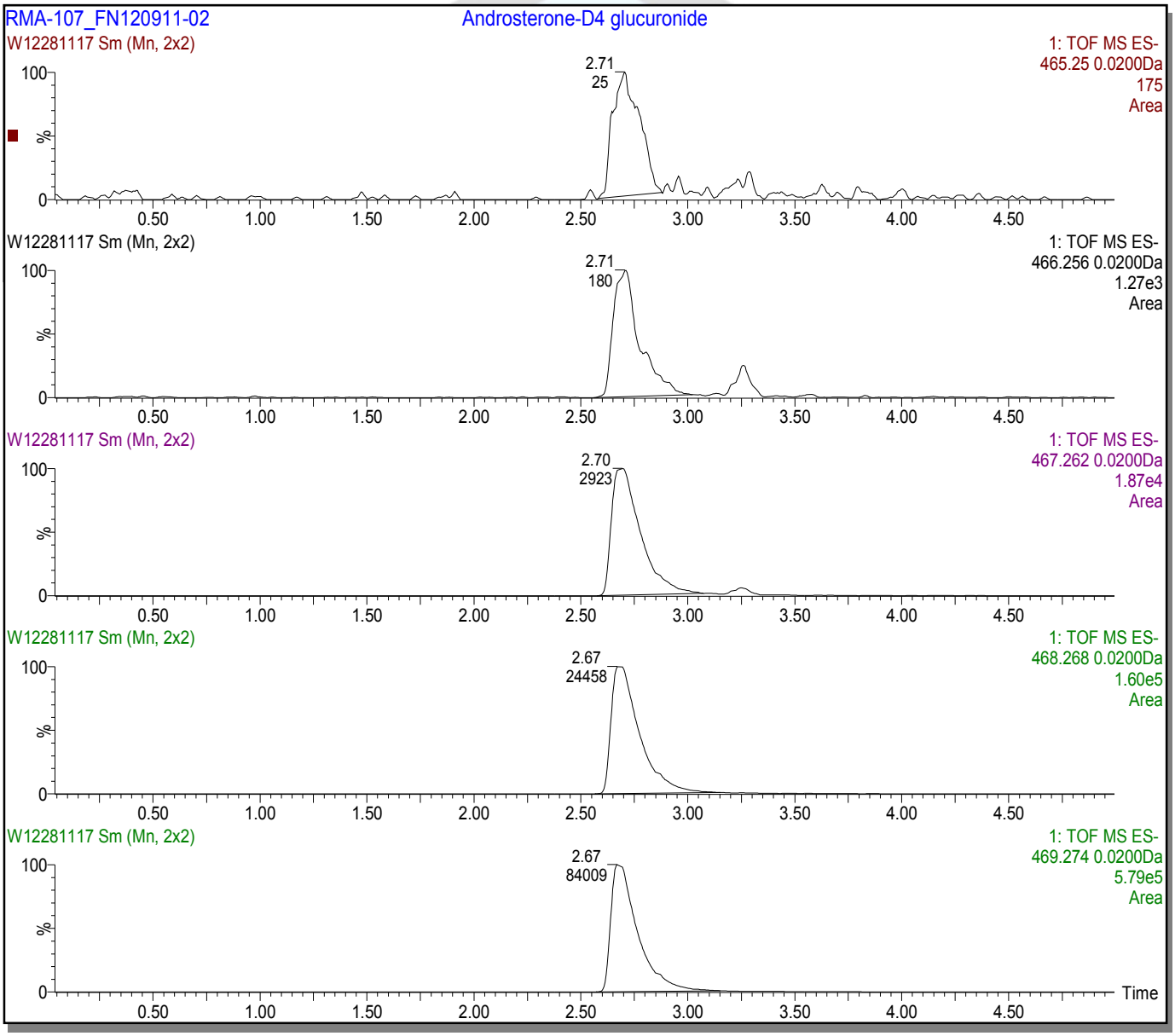
Column:	Zorbax Eclipse Plus C18 RRHD 1.8µ, 2.1 x 50 mm
Mobile Phase:	0.1% Formic acid in Water::Acetonitrile (80::20 to 40::60 at 2.5 mins, to 80::20 at 4.1 mins, hold 3.9 mins)
Flow Rate:	0.4 mL/min
Scan Range:	50-1200 amu
Ionization:	Electrospray, Negative Ion
Data File Name:	W12281117
Instrument:	Waters XEVO G2 QTOF
Sample Name:	FN120911-02
Acquired:	December 28, 2011



Spectral and Physical Data (cont.)

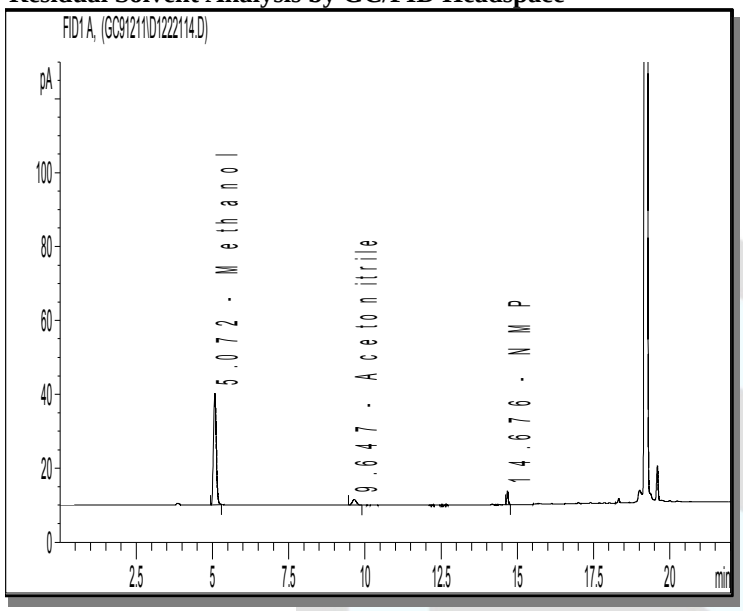
Isotopic Purity by LC/MS SIM Analysis

Column:	Zorbax Eclipse Plus C18 RRHD 1.8µ, 2.1 x 50 mm	**Data File Name:**	W12281117
Mobile Phase:	0.1% Formic acid in Water::Acetonitrile (80::20 to 40::60 at 2.5 mins, to 80::20 at 4.1 mins, hold 3.9 mins)	**Operator:**	JDC
		Instrument:	Waters XEVO G2 QTOF
		Sample Name:	FN120911-02
Flow Rate:	0.4 mL/min	**Method File:**	28-64C3
Scan Range:	465-469 amu	**Acquired:**	December 28, 2011
Ionization:	Electrospray, Negative Ion		



Spectral and Physical Data (cont.)

Residual Solvent Analysis by GC/FID Headspace

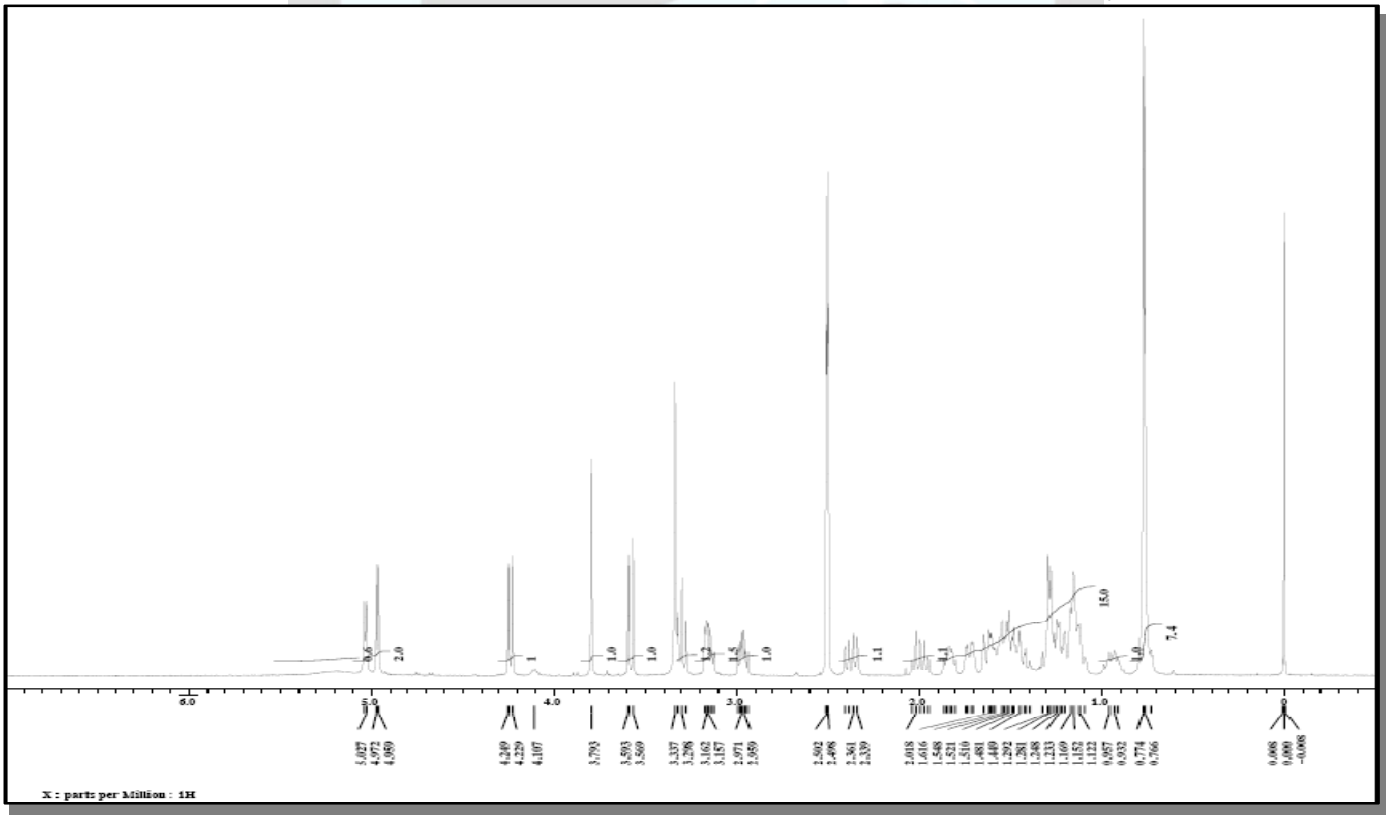


Column:	DB-ALC1 30 m x 0.53 mm, 3 µm film thickness
Temp Program:	40°C (12 min) to 220°C at 40°C/min (5.5 min)
Carrier Gas:	Helium
Flow Rate:	2.0 mL/min
Detector Heater Temp:	250°C
Injector:	Headspace Sampler
HS Oven Temp:	60°C
Vial Equilibration:	10 minutes
Data File Name:	C:\CHEM32\1\DATA\GC91211\D1222114.D
Instrument:	GC#9
Sample Name:	FN120911-02
Acquired:	December 22, 2011

Peak	Compound	Area	Weight %
1	Methanol	194.41	1.35
2	Acetonitrile	15.22	0.07
3	NMP	NA	NA
Total			**1.42**

^{1}H NMR

Instrument: JEOL ECS 400
Solvent: DMSO-D_6



Stability

***Short Term Stability** : A summary of accelerated stability findings for this product is listed below.*		
Storage Condition	**Mean Kinetic Temperature (MKT)**	**Time Period**
Freezer	-15°C	No decrease in purity was noted after one week.
Refrigerator	4°C	No decrease in purity was noted after one week.
Room Temperature	21°C	No decrease in purity was noted after one week.
40°C	40°C	~ 1% decrease in purity was noted after one week
***Transport/Shipping** : Stability data supports transport of this product at MKT not exceeding 4°C over a period of one week.*		
***Short Term Storage:** Stability data supports short term storage up to 3 months at Refrigerate conditions.*		
***Long Term Stability:** Long term stability has been assessed for freezer storage (-10 °C to -25 °C) conditions. Stability of a minimum of 25 months has been established through real-time stability studies.*		

COA Revision History

Revision No.	Date	Reason for Revision
00	February 3, 2012	Initial version
01	February 6, 2013	Revised Retest Date from March 2013 to March 2014.
02	April 15, 2014	Revised Retest Date from March 2014 to February 2015.