

# Certificate of Analysis

## Equilin

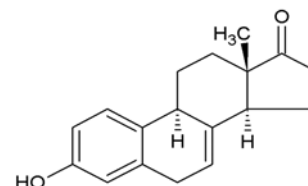
Cerilliant Quality

**ISO GUIDE 34**  
CERTIFICATE AR1353

**ISO/IEC 17025**  
CERTIFICATE AT1352

**ISO 9001:2008**  
CERTIFICATE 3854

**Catalog Number:** E-078  
**Solution Lot:** FN080411-02  
**Retest Date:** November 2014  
**Solvent:** Methanol  
**Volume per Ampule:** Not less than 1 mL  
**Storage:** Store in Freezer. See Stability Section.  
**Intended Use:** For R&D/ analytical purposes only. Not suitable for human or animal consumption.  
**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	Chromatographic Purity	Certified Concentration
Equilin	99.9%	1.000 ± 0.006 mg/mL
<ul style="list-style-type: none"> <li>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.</li> <li>Concentration is corrected for chromatographic purity, residual water, residual solvents and residual inorganics.</li> </ul>		

### Solution Standard Verification and Homogeneity

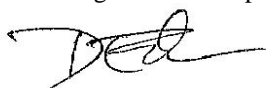
Standard Solution	Lot Number	Verified Concentration (mg/mL)		%RSD - Homogeneity	
		Actual Results	Acceptance Criteria	Actual Results	Acceptance Criteria
New Lot	FN080411-02	1.003	± 3%	0.8	≤ 3%
<ul style="list-style-type: none"> <li>Concentration is verified through multiple analyses and is calculated as the average of multiple analyses compared to an independently prepared calibration solution.</li> <li>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.</li> </ul>					

### 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.



  
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 Darron Ellsworth, Quality Assurance Manager

October 15, 2013

\_\_\_\_\_  
 Date

### Standard Solution Assay Parameters

**Analysis Method:** UV/Vis  
**Wavelength:** 281 nm  
**Slit Width:** 1.0 nm  
**Response:** 0.5 s

### Calibration Curve

**Calibration Curve:** Linear Regression  
**Number of Points:** 4  
**Linearity (r):** 1.000

### Neat Material Data

**Compound Name:** Equilin  
**Compound Lot:** PN071111-01

**Chemical Formula:** C<sub>18</sub>H<sub>20</sub>O<sub>2</sub>  
**CAS Number:** 474-86-2  
**Molecular Weight:** 268.35

#### Neat Material Characterization Summary

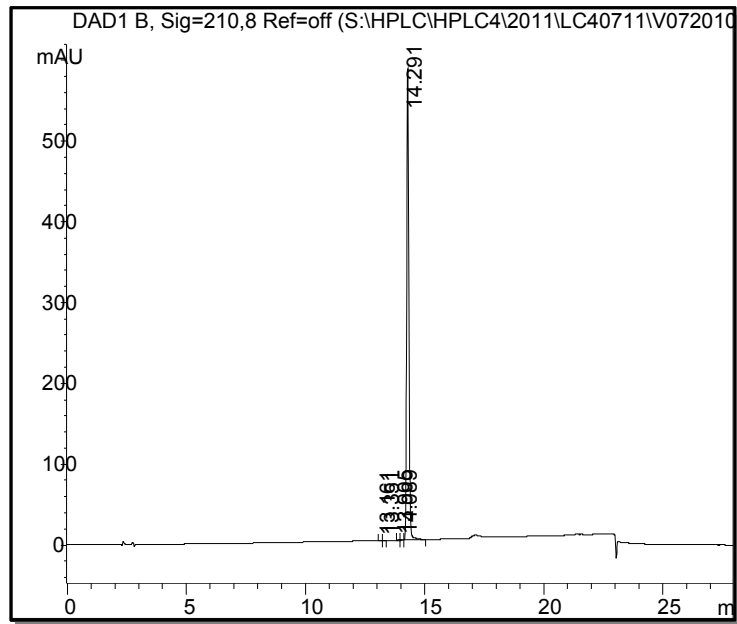
Analytical Test	Method	Results
Primary Chromatographic Purity by HPLC/PDA Analysis	SP10-0102	99.9%
Secondary Chromatographic Purity by GC/FID Analysis	SP10-0101	99.6%
Identity by GC/MS Analysis	SP10-0105	Consistent with Structure
Identity by <sup>1</sup> H-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure
Residual Solvent Analysis by GC/FID Headspace	AM1087 <sup>1</sup>	None Detected
Residual Water Analysis by Karl Fischer Coulometry	USP <921>, SP10-0103	0.07%
Inorganic Content by Microash Analysis	SP10-0135	< 0.2%
Purity Factor		99.84%

- ♦ Primary 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.
- ♦ The primary chromatographic purity value is used to calculate the Purity Factor.
- ♦ A secondary chromatographic purity method is utilized as a control.
- ♦ Purity Factor = [(100 - wt% residual solvent - wt% residual water - wt% residual inorganics) x Chromatographic Purity/100].
- ♦ Purity factor does not include adjustment for chiral and/or isotopic purity.

<sup>1</sup> Validated analytical method

Spectral and Physical Data

HPLC/PDA



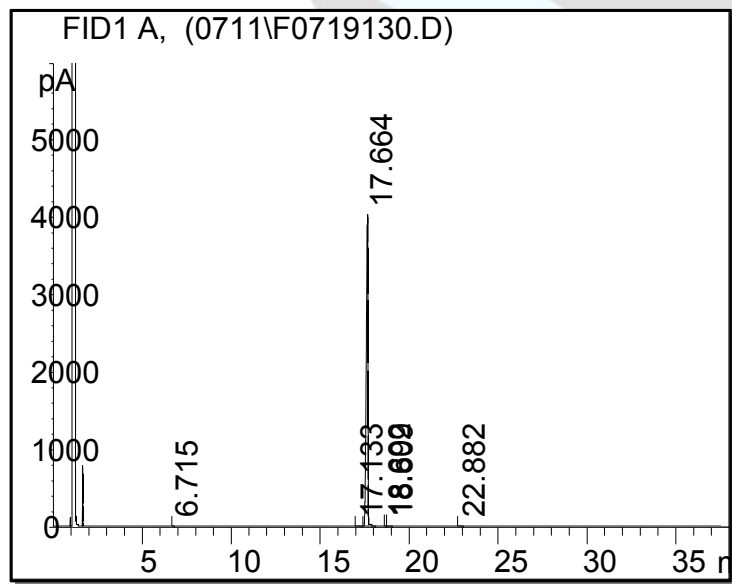
**Column:** Eclipse XDB C18 5 $\mu$ , 4.6 x 250 mm  
**Mobile Phase:** A: Acetonitrile B: Water  
**Gradient**

Time (mins)	%A	%B
0	20	80
20	90	10
20.1	20	80

**Program:**  
**Flow Rate:** 1.0 mL/min  
**Wavelength:** 210 nm  
**Data File Name:** S:\HPLC\HPLC4\2011\LC40711\V0720108.D  
**Operator:** TNT  
**Instrument:** LC#4  
**Sample Name:** PN071111-01  
**Method File:** PRM252-1.M  
**Acquired:** July 20, 2011 11:55 AM

Peak #	Ret Time	Area	Height	Area %
1	13.16	0.50	0.08	0.01
2	13.35	0.36	0.03	0.01
3	13.91	0.77	0.15	0.02
4	14.09	1.66	0.34	0.05
5	14.29	3605.51	584.22	99.91

GC/FID

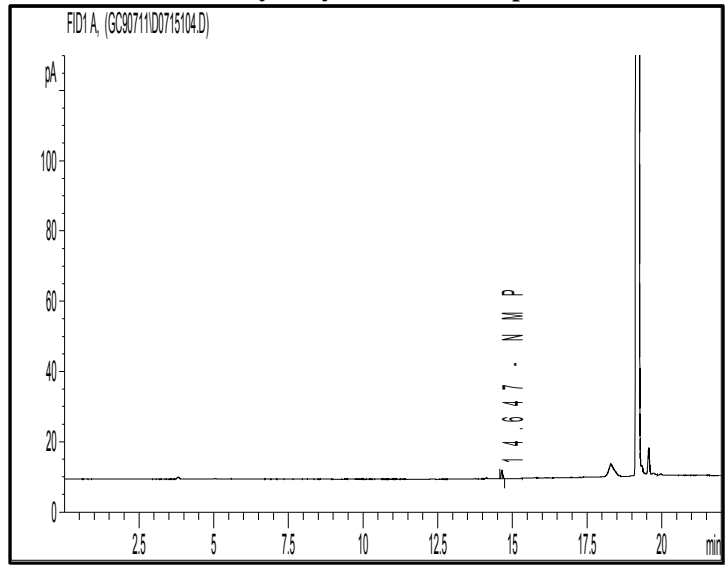


**Column:** DB-5ms, 30 m x 0.53 mm ID, 1.5  $\mu$ m film thickness  
**Temp Program:** 40°C to 200°C at 40°C/min  
 200°C to 280°C at 5°C/min (hold 18 min)  
**Injector Temp:** Cool-on-Column  
**Detector Temp:** 325°C  
**Data File Name:** S:\GC\GC6\2011\0711\F0719130.D  
**Operator:** PT  
**Instrument:** GC#6  
**Sample Name:** PN071111-01  
**Method File:** B01E.M  
**Acquired:** July 20, 2011 1:49 AM

Peak #	Ret Time	Area	Height	Area %
1	6.72	3.31	1.09	0.01
2	17.13	8.53	0.67	0.04
3	17.66	23872.80	4003.25	99.65
4	18.69	26.22	3.66	0.11
5	18.81	38.22	5.45	0.16
6	22.88	7.75	1.10	0.03

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

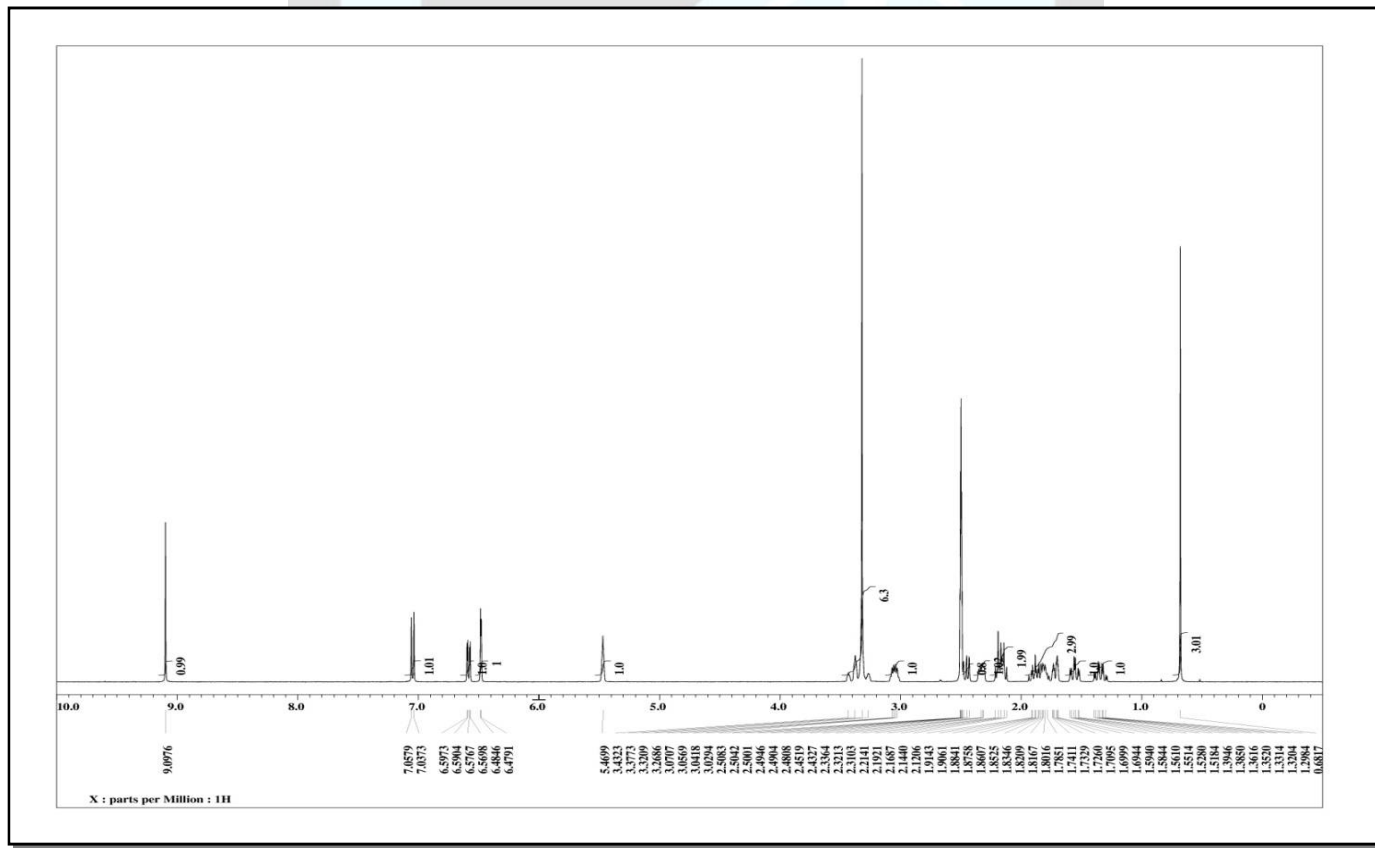
**Data File Name:** C:\HPCHEM\1\DATA\GC90711\D0715104.D  
**Operator:** RPC  
**Instrument:** GC#9  
**Sample Name:** PN071111-01  
**Acquired:** July 15, 2011 11:42 AM

Peak	Compound	Area	Weight %
1	NMP	NA	NA
<b>Total</b>			<b>ND</b>

ND - Not Detected

<sup>1</sup>H NMR

**Instrument:** JEOL ECS 400  
**Solvent:** DMSO-D<sub>6</sub>



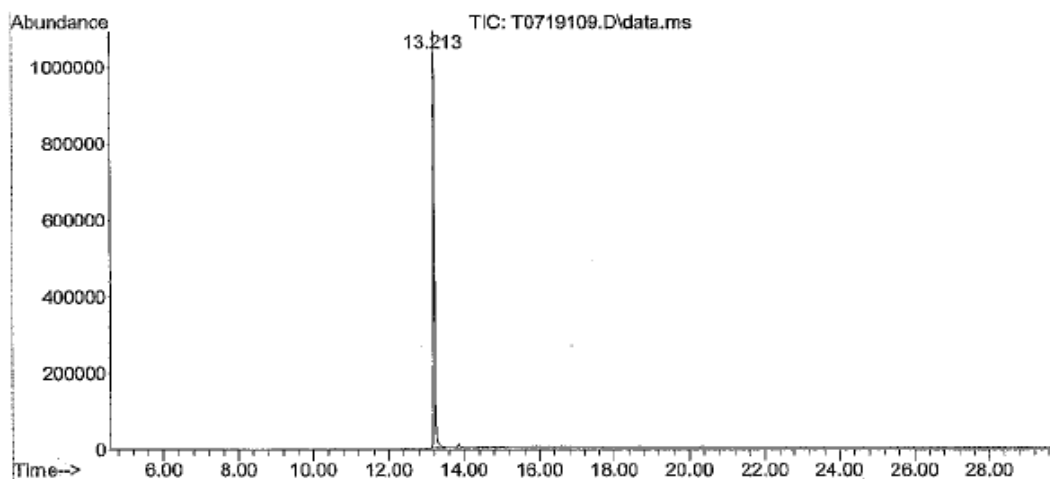
Spectral and Physical Data (cont.)

Identity by GC/MS Analysis

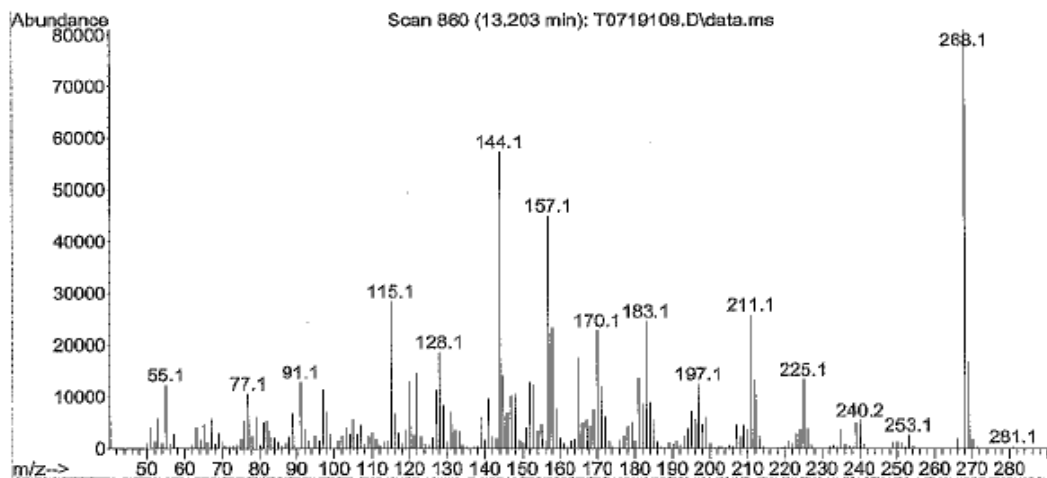
Compound Name : Equilin  
Lot Number : PN0711111-01  
Instrument : Agilent 5973N MSD/6890N GC  
Operator-Inst ID : BD - C10106  
Date Reported : Wed Jul 20 08:45:29 2011  
Column Type : DB-5ms, 30m x 0.25mm ID, 0.25um film thickness  
Temp. Program : 50°C to 200°C @ 10°C/min, 200°C to 300°C @ 10°C/min, 16min hold  
Injector Temp. : Cool on-column  
Carrier Gas : Helium  
Flow Rate (mL/min) : 0.80 mL/min  
Transfer Line Temp. : 280°C

Scan Range : 50-500

Total Ion Chromatogram



Mass Spectrum



## Stability

**Short Term Stability :** A summary of accelerated stability findings for the related product E-075 Estrone 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	
Room Temperature	21°C	
40°C	40°C	

**Transport/Shipping :** Stability data supports transport of this product at MKT not exceeding 40 °C over a period of one week.

## COA Revision History

Revision No.	Date	Reason for Revision
00	August 11, 2011	Initial version
01	August 20, 2012	Revised Retest Date from October 2012 to October 2013.
02	October 15, 2013	Revised Retest Date from October 2013 to November 2014.