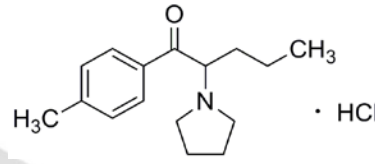
Cerilliant
Analytical Reference Standards
a SIGMA-ALDRICH company

Certified Reference Material - Certificate of Analysis

Pyrovalerone , Primary Standard

Cerilliant Quality
ISO GUIDE 34
ISO/IEC 17025
ISO 13485
ISO 15194
ISO 9001
GMP/GLP



Catalog Number: P-081
Lot: FE01261505
Retest Date: February 2020
Description: Pyrovalerone HCl in Methanol.
Nominal concentration is adjusted for HCl content.
Packaging: Solution in 2 mL amber USP Type I glass ampoule containing not less than 1 mL of certified solution.
Storage: Store unopened in freezer (-10 °C to -25 °C).
Shipping: Ambient. See Stability Section.
Intended Use: This Certified Reference Material is suitable for the *in vitro* identification, calibration, and quantification of the analyte(s) in analytical and R&D applications. Not suitable for human or animal consumption.
Instructions for Use: Users should quantitatively transfer desired volume using established good laboratory practices to spike into matrix or to dilute to the desired concentration. Each ampoule is intended for one-time use.
Regulatory: USDEA Exempt | Canadian TK # 61-654 **Safety: Danger. See Safety Data Sheet**

- Retest Date - stability studies ongoing. Certificate of Analysis will be updated upon completion of retest.
- Ampoules are overfilled to ensure a minimum 1 mL volume can be transferred when using a 1 mL Class A volumetric pipette.
- For quantitative applications, the minimum sample size for intended use is 1 µL.

Analyte	Certified Concentration Value
Pyrovalerone	1.000 ± 0.005 mg/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 mass balance purity factor, material density, balance, and weighing technique.
- This standard is prepared gravimetrically and mass results are reported on the conventional basis for weighing in air. Nominal concentration is calculated based on: the actual measured mass; Mass Balance 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. Nominal concentration is adjusted for HCl content. No adjustment required before use.
- Additional certification information available upon request.

Metrological Traceability

- This standard has been prepared and certified under the ISO Guide 34, ISO/IEC 17025, ISO 9001 and ISO 13485 standards. This standard meets the requirements of a Certified Reference Material and a Primary Standard as defined by ISO and is traceable to the SI and higher order standards through an unbroken chain of comparisons.
- This standard has been gravimetrically prepared using balances that have been fully qualified and calibrated to ISO 17025 requirements. All calibrations utilize NIST traceable weights which are calibrated externally by a qualified ISO 17025 accredited calibration laboratory to NIST standards. Qualification of each balance includes the assignment of a minimum weighing by a qualified and ISO 17025 accredited calibration vendor taking into consideration the balance and installed environmental conditions to ensure compliance with USP tolerances of NMT 0.1% relative error. Balance calibration adjustments are performed weekly utilizing the balance's internal adjustment mechanism. Calibration verifications are performed pre-use. Weigh tapes from the calibration verification are included in the production batch record for this standard. Production data package available upon request.
- Fill volume is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- Concentration is verified against an independently prepared calibration solution gravimetrically prepared.
- Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques. Spectral data is provided on subsequent pages of this COA. The density and material Mass Balance Purity Factor is traceable to the SI and higher order reference standards through mass measurement and instrument qualification and calibrations.

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.

ACCLASS ACCREDITED
REFERENCE MATERIAL PRODUCER

Darron Ellsworth, Quality Assurance Manager

August 21, 2015
Date

Solution Standard Verification

Concentration accuracy and within- and between-bottle homogeneity are analytically verified against an independently prepared calibration solution and to the prior lot.

Solution standard verification demonstrates confirmation that the specified requirements for the Primary Standard have been fulfilled and validated under ISO 13485.

Standard Solution Assay Parameters

Analysis Method: HPLC/UV
Column: Ascentis Express Phenyl-Hexyl, 2.7 µm, 3.0 x 50 mm
Mobile Phase: A:: Acetonitrile
B:: 0.1% Phosphoric acid in Water

Gradient:

Time (min)	% A	% B
0.00	90	10
0.10	90	10
2.00	50	50
3.50	50	50
3.51	90	10

Flow Rate: 1.0 mL/min
Wavelength: 254 nm

Calibration Curve

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

Standard Solution	Lot Number	Verified Concentration (mg/mL) Actual Results	Verified Concentration (mg/mL) Acceptance Criteria	%RSD - Homogeneity Actual Results	%RSD - Homogeneity Acceptance Criteria
New Lot	FE01261505	1.016	± 3%	0.5	≤ 3%
Previous Lot	FN082411-03	1.019	± 3%	0.7	≤ 3%

- Concentration is verified through multiple analyses and is calculated as the average of multiple analyses compared to an independently prepared calibration solution.
- Within-sample and between-sample homogeneity of the New Lot is ensured through rigorous production process controls statistically analyzed to evaluate risk and verified by analysis. Multiple samples pulled from across the lot using a random stratified sampling plan were analyzed to verify homogeneity. % RSD results shown above for the New Lot demonstrate ampoule-to-ampoule homogeneity.

Analyte Certification - Mass Balance Purity Factor

Each analyte is thoroughly identified and characterized using an orthogonal approach. A mass balance purity factor is assigned incorporating chromatographic purity and residual impurities. The mass balance purity factor and salt adjustment are utilized to calculate the weighing adjustment necessary to ensure accuracy of the solution standard concentration.

Material	Lot Number	CAS Number	Chemical Formula	Molecular Weight (salt)	Molecular Weight (base)	Salt Adjustment
Pyrovalerone HCl	FC072811-01	1147-62-2	$C_{16}H_{23}NO \cdot HCl$	281.82	245.36	1.149

Material Characterization Summary

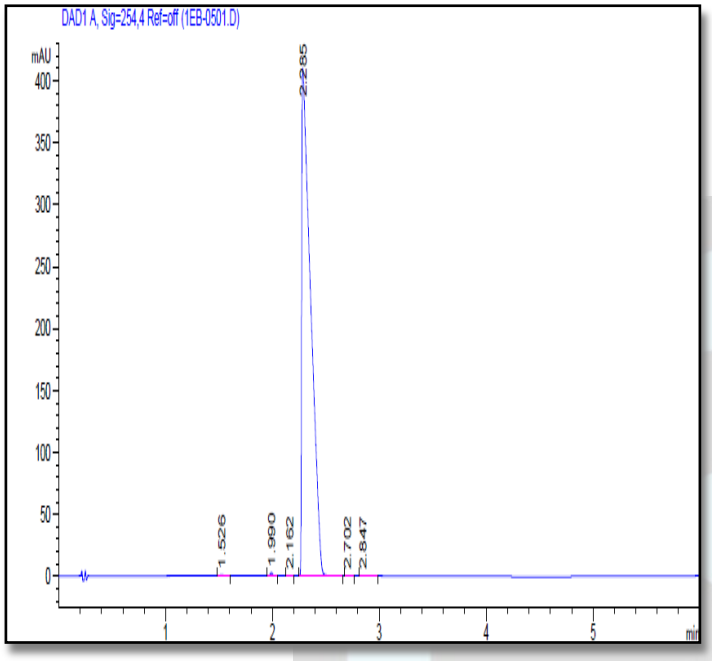
Analytical Test	Method	Results
Primary Chromatographic Purity by HPLC/UV Analysis	SP10-0102	99.8%
Secondary Chromatographic Purity by GC/FID Analysis	SP10-0101	99.5%
Identity by LC/MS Analysis	SP10-0107	Consistent with Structure
Identity by ^{1}H-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure
Residual Solvent Analysis by GC/FID Headspace	AM1087 [1]	0.04%
Residual Water Analysis by Karl Fischer Coulometry	AM1346 [1]	0.99%
Inorganic Content by Microash Analysis	SP10-0135	< 0.2%
Mass Balance Purity Factor		98.73%

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

[1] Validated analytical method

Spectral and Physical Data

HPLC/UV



Column: Ascentis Express Phenyl-Hexyl, 2.7 µm, 3.0 x 50 mm
Mobile Phase: A:: Acetonitrile
B:: 0.1% Phosphoric acid in Water
Gradient:

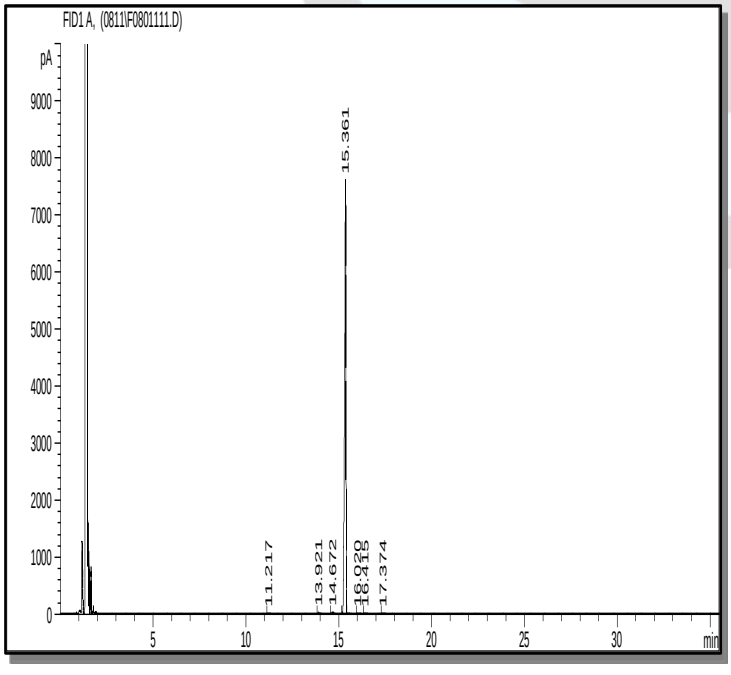
Time (min)	%A	%B
0.00	10	90
0.10	10	90
2.00	30	70
3.50	30	70
3.51	10	90

Flow Rate: 1.0 mL/min
Wavelength: 254 nm

Data File Name: RMP-117 P LC 14 2015-01-07 20-00-52\1EB-0501.D
Instrument: LC#14
Sample Name: FC072811-01
Acquired: January 07, 2015

Peak #	Ret Time	Area	Height	Area %
1	1.53	1.46	0.84	0.06
2	1.99	3.29	2.48	0.14
3	2.16	0.30	0.15	0.01
4	2.29	2359.90	409.65	99.76
5	2.70	0.23	0.13	0.01
6	2.85	0.44	0.20	0.02

GC/FID



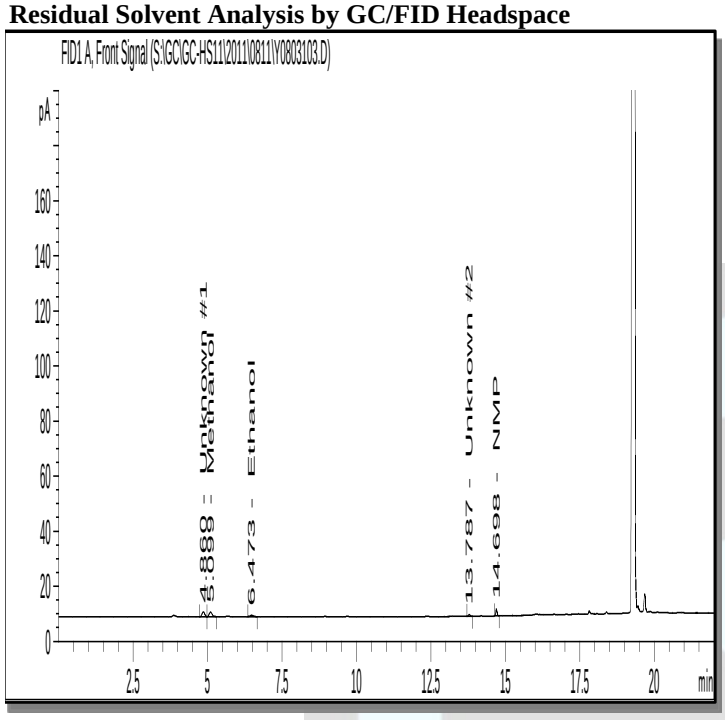
Column: DB-5ms, 30 m x 0.53 mm ID, 1.5 µm film thickness
Temp Program: 40°C to 140°C at 40°C/min
140°C to 280°C at 5°C/min hold 5 min
Injector Temp: Cool-on-Column
Detector Temp: 325°C

Data File Name: S:\GC\GC6\2011\0811\F0801111.D
Instrument: GC#6
Sample Name: FC072811-01
Acquired: August 02, 2011

Peak #	Ret Time	Area	Height	Area %
1	11.22	14.53	3.62	0.04
2	13.92	43.47	10.04	0.11
3	14.67	105.77	23.81	0.28
4	15.36	37885.40	7606.09	99.54
5	16.02	5.44	0.80	0.01
6	16.42	1.82	0.39	0.00
7	17.37	4.43	0.90	0.01

Spectral and Physical Data (cont.)

Residual Solvent Analysis by GC/FID Headspace



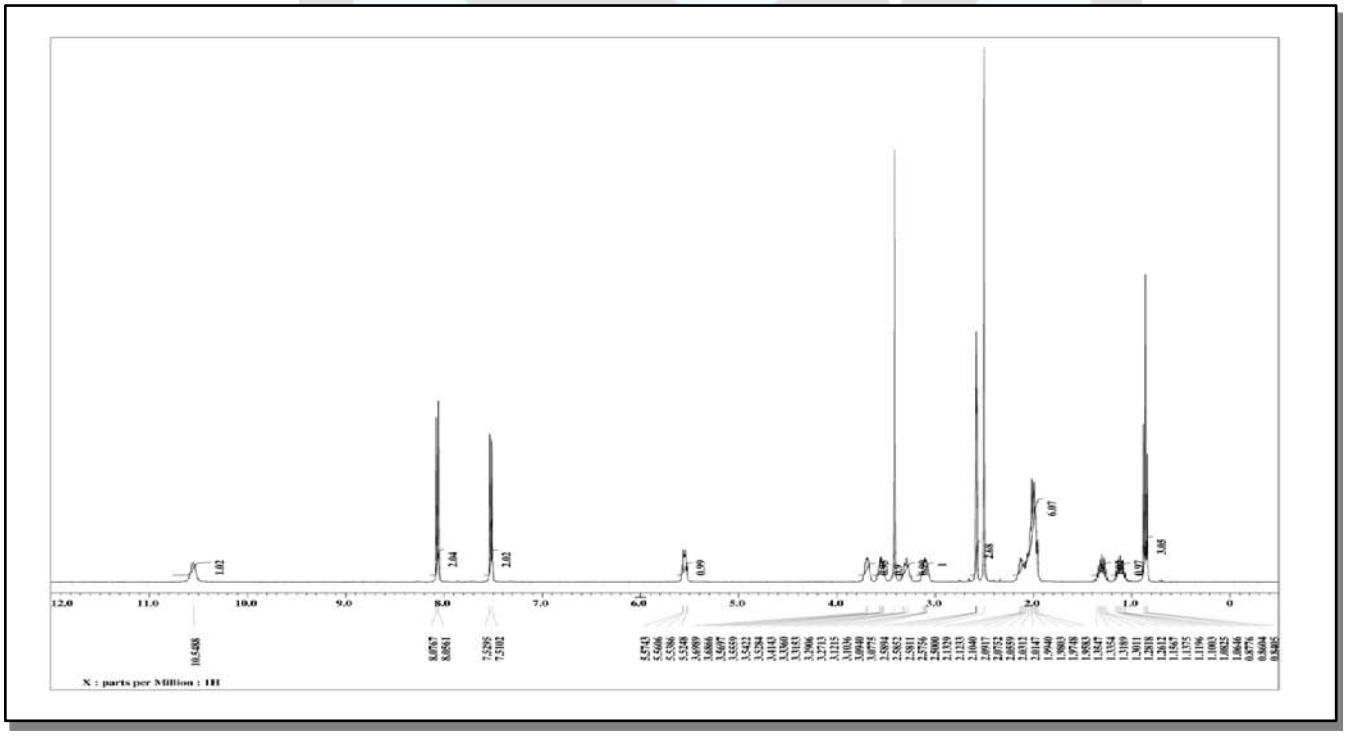
Column:	DB-ALC1 30 m x 0.53 mm, 3 µm film thickness
Temp Program:	40°C (12 min) to 220°C at 40°C/min (5.5 min)
Carrier Gas:	Helium
Flow Rate:	2.0 mL/min
Detector Heater Temp:	250°C
Injector:	Headspace Sampler
HS Oven Temp:	60°C
Vial Equilibration:	10 minutes
Data File Name:	S:\GC\GC-HS11\2011\0811\Y0803103.D
Instrument:	GC#11
Sample Name:	FC072811-01
Acquired:	August 03, 2011

Peak	Compound	Area	Weight %
1	Unknown # 1	10.79	BQL
2	Methanol	10.98	0.04
3	Ethanol	3.62	BQL
4	Unknown # 2	2.69	BQL
5	NMP	NA	NA
Total			**0.04**

BQL - Below Quantitation Limit

^{1}H NMR

Instrument:	JEOL ECS 400
Solvent:	DMSO-D_6



Spectral and Physical Data (cont.)

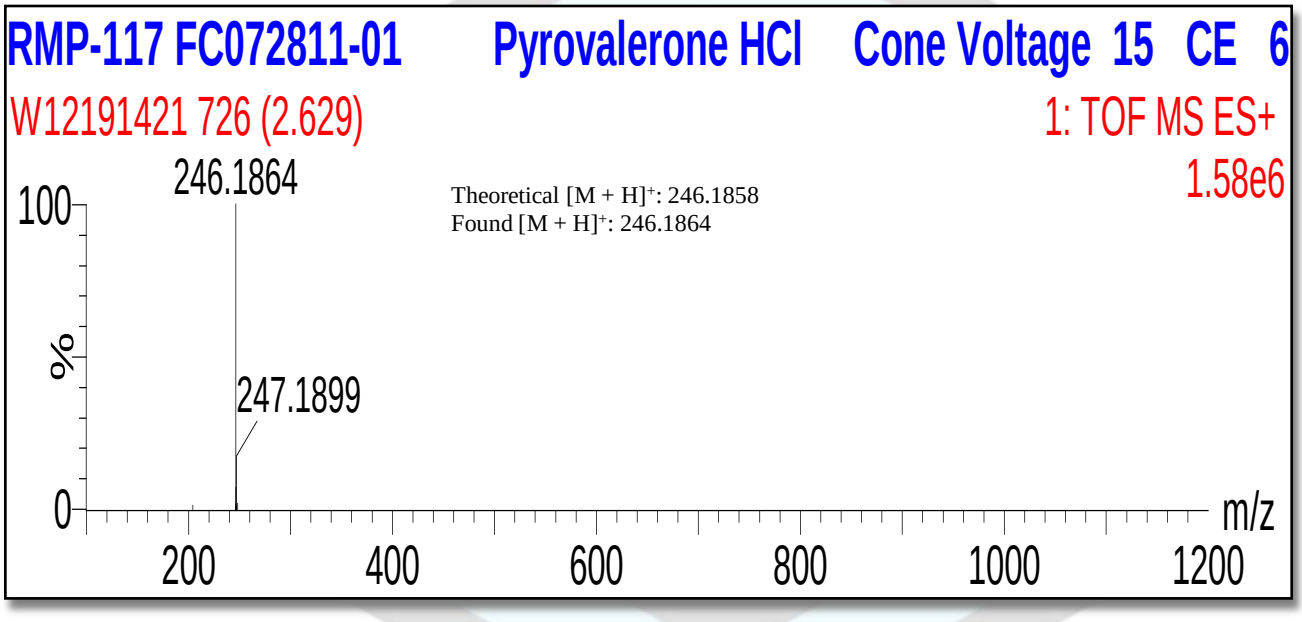
LC/MS

Column: Ascentis Express C18, 2.7 µm, 3.0 x 50 mm
Mobile Phase: A:: 0.1% Formic acid in Water
B:: Acetonitrile

Gradient:

Time (min)	% A	% B
0.0	90	10
0.5	90	10
4.0	50	50
5.8	50	50
6.0	90	10
8.0	90	10

Flow Rate: 0.4 mL/min
Scan Range: 100 - 1200 amu
Ionization: Electrospray, Positive Ion
Data File Name: W12191421
Instrument: Waters XEVO G2 QTOF
Sample Name: FC072811-01
Acquired: December 19, 2014



Stability

Short term stability studies have been performed under accelerated conditions for a period of up to four weeks. Short term data is utilized to predict long term stability and to support transport conditions and normal laboratory use. Real-time stability studies are performed at the recommended storage conditions over the life of the product.

Short Term Stability: *A summary of accelerated stability findings for this product is listed below.*		
Storage Condition	**Mean Kinetic Temperature (MKT)**	**Time Period/Result**
Freezer	-15°C	No decrease in purity was noted after four weeks.
Refrigerator	4°C	
Room Temperature	21°C	
40°C	40°C	
Transport/Shipping: *Stability studies support the transport of this product at ambient conditions.*		
Short Term Storage: *Stability data supports short term storage for no more than 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 45 months has been established through real-time stability studies.*		

COA Revision History

Revision No.	Date	Reason for Revision
00	August 21, 2015	Initial version