

Certified Reference Material - Certificate of Analysis

Benzylfentanyl, Primary Measurement Standard

N-(1-Benzylpiperidin-4-yl)-N-phenylpropanamide HCl

Product No.: B-085-1ML
Lot No.: FN02011902
Description of CRM: Benzylfentanyl HCl in Methanol (Solution)
 Nominal concentration is adjusted for HCl content.
Retest Date: August 2022 See Stability Section
Storage: Store unopened in freezer (-10 °C to -25 °C).
Shipping: Ambient. See Stability Section
Chemical formula: C₂₁H₂₆N₂O•HCl
CAS No.: 5156-58-1

Cerilliant Quality

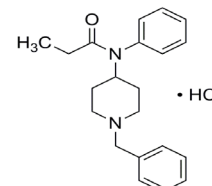
ISO 17034

ISO/IEC 17025

ISO 13485

ISO 14001

ISO 9001



Analyte	Certified Concentration ± associated uncertainty U, u=k*u (k=2)
Benzylfentanyl	1.000 ± 0.006 mg/mL

Metrological traceability: Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. See "Details on metrological traceability" on page 3.

Measurement method: The certified value is calculated from high precision weighing of thoroughly characterized starting material. See "Details about certification process" on page 3.

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.

Minimum sample size: 1 µL for quantitative applications

Instructions for handling and correct use: Concentration is corrected for chromatographic purity, residual water, residual solvents, and residual inorganics. No adjustment required before 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. Nominal concentration is adjusted for HCl content. No adjustment required before use.

Health and safety information: Danger. Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.

Accreditation: Cerilliant Corp. is accredited by the US accreditation authority ANAB as registered reference material producer AR-1353 in accordance with ISO 17034 and registered testing laboratory AT-1352 according to ISO/IEC 17025.




Darron Ellsworth, Quality Assurance Manager

August 23, 2021

Issue Date

Packaging:

2 mL amber USP Type 1 glass ampoule containing not less than 1 mL of certified solution. Ampoules are overfilled to ensure a minimum of 1 mL volume can be transferred when using a 1mL Class A volumetric pipette.

Details on starting materials:

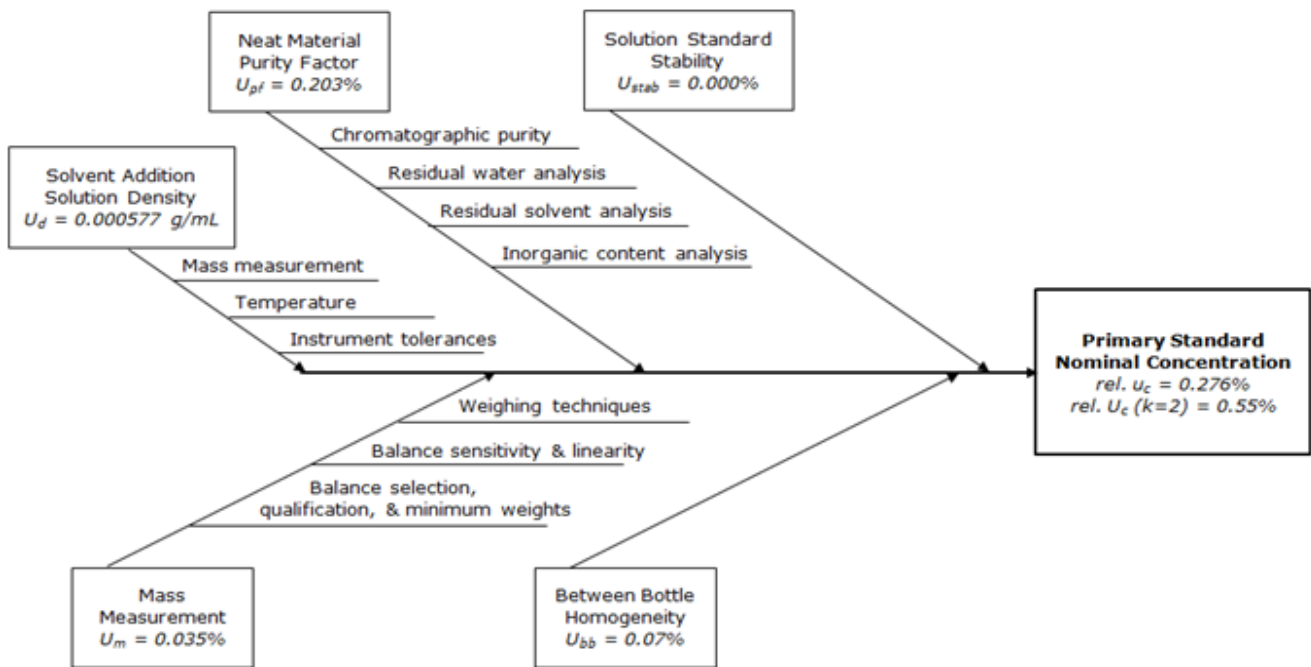
Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques and is assigned a Mass Balance Purity Factor. Spectral data is provided on subsequent pages of this CoA.

Certificate of Origin:

Cerilliant Corporation certifies no material of animal origin (BSE/TSE) was used in the preparation of this product. This material was manufactured in the USA.

Associated uncertainty:

The uncertainty has been calculated by statistical analysis of all aspects of our production system and incorporated uncertainty of the mass balance purity factor, material density, balance, weighing technique, and homogeneity. Uncertainty components of the gravimetrically prepared Primary Standard concentration are shown in the figure below. Uncertainty is expressed as an expanded uncertainty in accordance with ISO 17034 at the approximate 95% confidence interval using a coverage factor of k=2. Uncertainty contribution from neat material homogeneity was established to be negligible through establishment of process controls and verification of the control process. Stability uncertainty was determined to be negligible by regression analysis.



Details on metrological traceability:

- ♦ 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 0.10% relative error.
- ♦ Fill volume to predetermined specifications is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- ♦ The density and material Mass Balance Purity Factor of each raw material is traceable to the SI and higher order reference materials through mass measurement and instrument qualification and calibrations.

Details about certification process:

This standard has been prepared and certified under the ISO 17034, 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.

- ♦ Nominal concentration is calculated based on: the actual mass; Mass balance purity factor of the analyte(s); measured mass of the solution; and the density of the pure diluent at 20°C.
- ♦ 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.
- ♦ Additional certification information available upon request.

Solution Standard Verification

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

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

Standard Solution Assay Parameters		Calibration Curve	
Analysis Method:	HPLC/UV	Calibration Curve:	Linear Regression
Column:	Ascentis Express C18, 2.7 µm, 3.0 x 100 mm	Number of Points:	4
Mobile Phase:	Acetonitrile:0.1% Phosphoric acid in Water (30:70)	Linearity (r) :	1.000
Flow Rate:	1.5 mL/min		
Wavelength:	210 nm		
		Verified Concentration (mg/mL)	%RSD - Homogeneity
Standard Solution	Lot Number	Actual Results	Actual Results
New Lot	FN02011902	1.008	0.5
<ul style="list-style-type: none"> 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 Name:	Benzylfentanyl HCl	Molecular Weight (base):	322.44
Material Lot:	FC10171802	Molecular Weight (salt):	358.90
Chemical Formula:	C ₂₁ H ₂₆ N ₂ O•HCl	Salt Adjustment:	1.113
CAS Number:	5156-58-1		

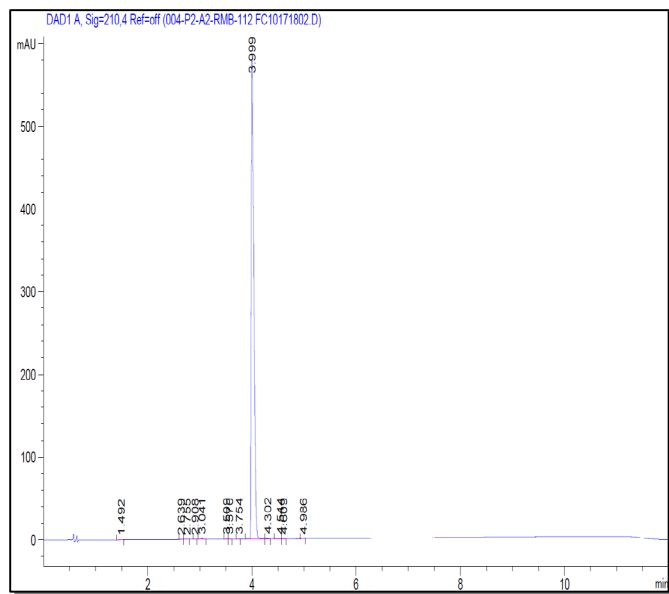
Material Characterization Summary				
Analytical Test	Method	Results		
Primary Chromatographic Purity by HPLC/UV Analysis	SP10-0102	99.6%		
Secondary Chromatographic Purity by GC/FID Analysis	SP10-0107	99.6%		
Identity by LC/MS Analysis	SP10-0107	Consistent with Structure		
Identity by ¹ H-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure		
Residual Solvent Analysis by GC/FID Headspace	AM1087 ¹	None Detected		
Residual Water Analysis by Karl Fischer Coulometry	AM1346 ¹	Below Quantitation Limit		
Inorganic Content by Microash Analysis	SP10-0135	< 0.2%		
Elemental Analysis	Outsourced	Calculated	Analyzed	
		C	70.28%	70.43%
		H	7.58%	7.67%
		N	7.81%	8.06%
Mass Balance Purity Factor		99.65%		

¹ Validated analytical method

- ♦ 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 purity method was selected to optimize resolution of impurities while minimizing degradation of the analyte. Secondary purity methods with orthogonal detector capabilities from the primary purity method are used as controls to confirm an accurate purity value.
- ♦ 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.

Spectral and Physical Data

HPLC/UV



Column: Ascentis Express C18, 2.7 μ m,
3.0 x 100 mm

Mobile Phase: A: Acetonitrile
B: 0.1% Phosphoric acid in Water

Gradient:

Time (min)	% A	% B
0.0	10	90
8.0	70	30
10.0	70	30
10.1	10	90

Flow Rate: 0.7 mL/min

Wavelength: 210 nm

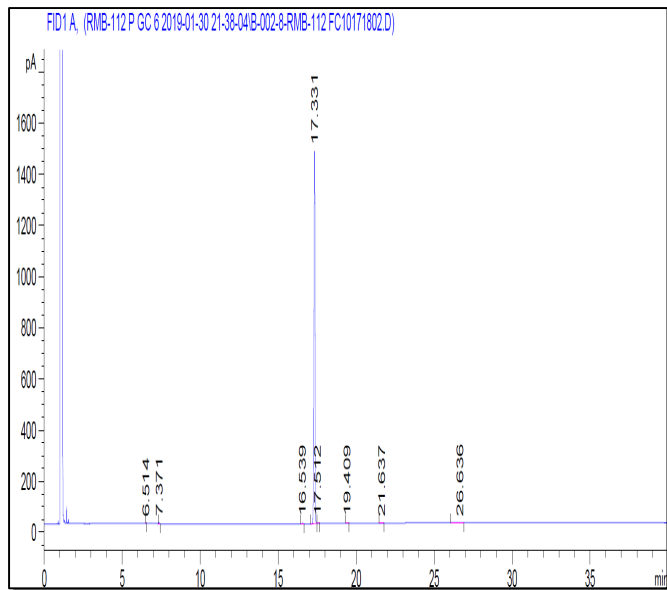
Sample Name: FC10171802

Acquired: January 25, 2019

Peak #	Ret Time	Area %
1	1.49	0.02
2	2.64	0.03
3	2.76	0.01
4	2.91	0.02
5	3.04	0.12
6	3.51	0.03
7	3.57	0.02
8	3.75	0.01
9	4.00	99.62
10	4.30	0.09
11	4.54	0.02
12	4.61	0.01
13	4.99	0.01

Spectral and Physical Data (cont.)

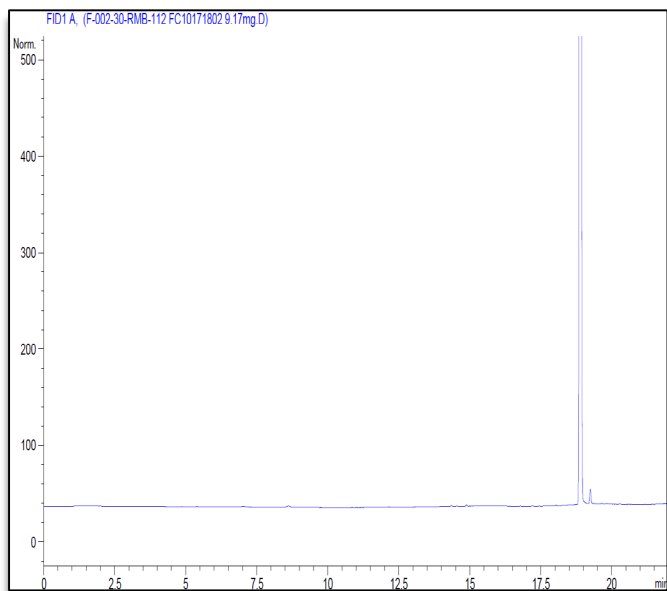
GC/FID



Column: DB-5ms, 30 m x 0.53 mm ID, 1.5 µm film thickness
Temp Program: 40°C to 200°C at 40°C/min
 200°C to 300°C at 5°C/min
 hold 16 min
Injector Temp: Cool-on-Column
Detector Temp: 325°C
Sample Name: FC10171802
Acquired: January 30, 2019

Peak #	Ret Time	Area %
1	6.51	0.00
2	7.37	0.20
3	16.54	0.02
4	17.33	99.63
5	17.51	0.03
6	19.41	0.01
7	21.64	0.06
8	26.64	0.05

Residual Solvent Analysis by GC/FID Headspace



Column: DB-ALC1 30 m x 0.53 mm, 3 µm film thickness
Temp Program: 40°C hold 12 min to 220°C at 40°C/min hold 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
Sample Name: FC10171802
Acquired: January 23, 2019

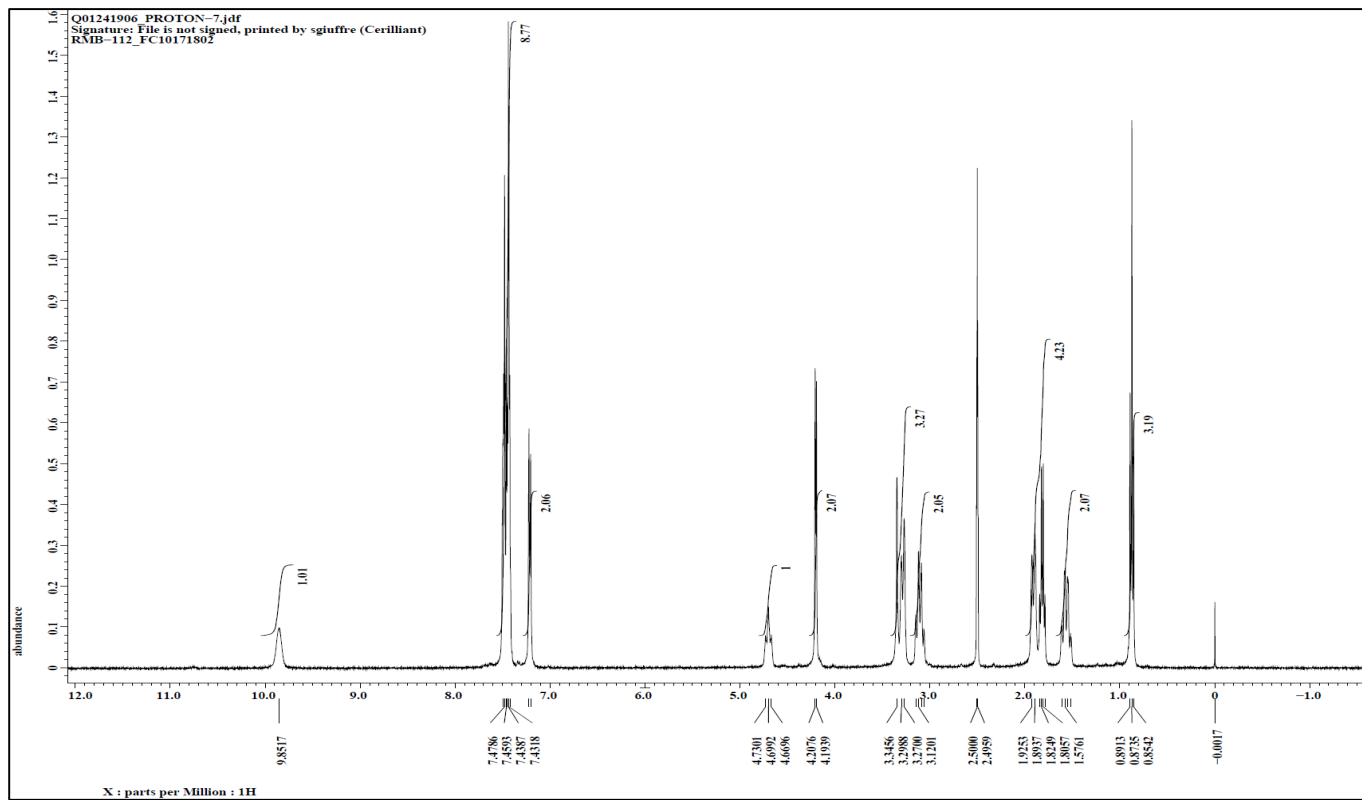
Peak	Compound	Area	Weight %
1	NMP	NA	NA
Total			ND

ND - None Detected

Spectral and Physical Data (cont.)

¹H NMR

Instrument: JEOL ECS 400
Solvent: DMSO-D₆



Spectral and Physical Data (cont.)

LC/MS

Column: Ascentis Express C18, 2.7 μ m,
3.0 x 50 mm

Mobile Phase: A: 0.1% Formic acid in Water
B: Acetonitrile

Gradient:

Time (min)	% A	% B
0.0	80	20
0.5	80	20
4.0	20	80
5.8	20	80
6.0	80	20
8.0	80	20

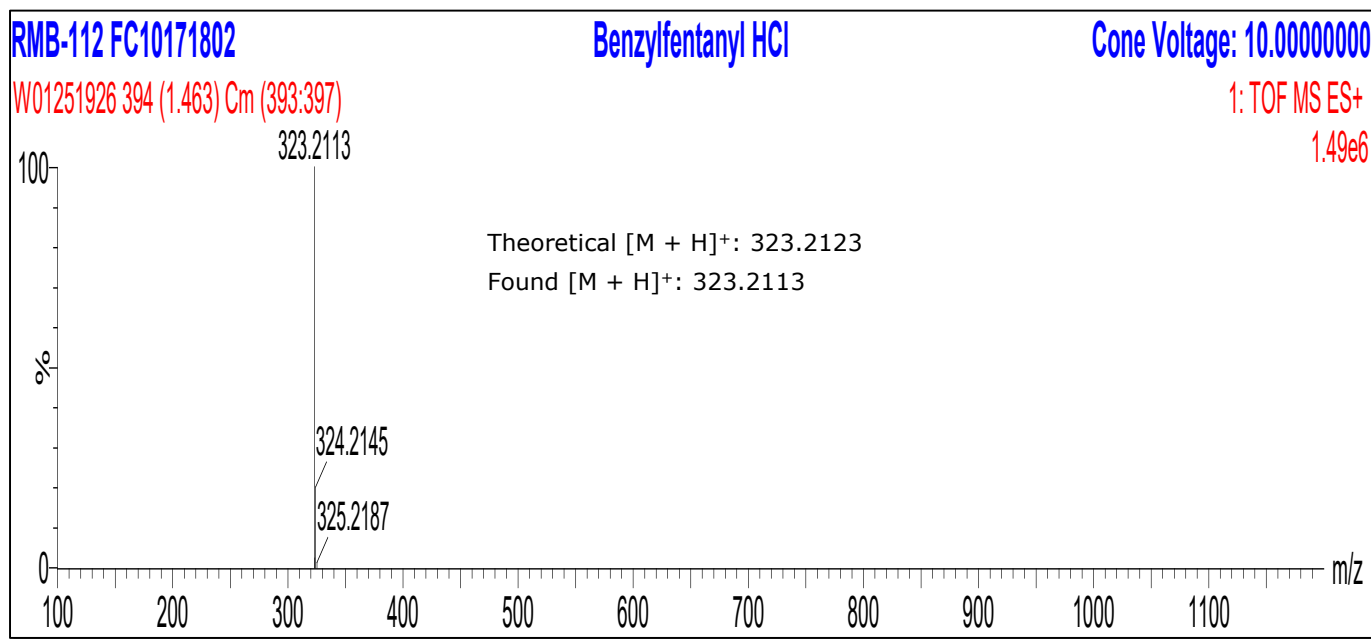
Flow Rate: 0.4 mL/min

Scan Range: 100-1200 amu

Ionization: Electrospray, Positive Ion

Instrument: Waters XEVO G2 QTOF

Acquired: January 25, 2019



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.

Long Term Stability: Long term stability has been assessed for Freezer storage (-10 °C to -25 °C) conditions. Stability of a minimum of 30 months has been established through real-time stability studies.

Commutability

This standard is a solution of a pure substance in an organic solvent and is a Primary Standard. This Primary Standard is suitable for use in the preparation of calibrators and/or controls in any biological matrix. This standard is not in a biological matrix and therefore commutability to methods or standards in biological matrices does not apply.

COA Revision History

Revision No.	Date	Reason for Revision
00	February 28, 2019	Initial version.
01	February 19, 2020	Revised Retest Date from April 2020 to February 2021.
		Added Long Term Stability Section.
02	December 14, 2020	Revised Retest Date from February 2021 to November 2021.
03	August 23, 2021	Revised Retest Date from November 2021 to August 2022.

Certified Reference Material - Certificate of Analysis

Benzylfentanyl-¹³C₆, Primary Measurement Standard

N-(1-Benzylpiperidin-4-yl)-*N*-phenylpropanamide-¹³C₆ HCl

Product No.: B-086-1ML
Lot No.: FN01211904
Description of CRM: Benzylfentanyl-¹³C₆ HCl in Methanol (Solution)
 Nominal concentration is adjusted for HCl content.
Retest Date: August 2022 See Stability Section
Storage: Store unopened in freezer (-10 °C to -25 °C).
Shipping: Ambient. See Stability Section
Chemical formula: C₁₅¹³C₆H₂₆N₂O•HCl
CAS No.: NA

Cerilliant Quality

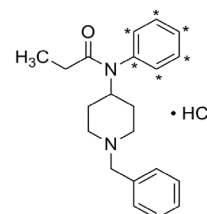
ISO 17034

ISO/IEC 17025

ISO 13485

ISO 14001

ISO 9001



Analyte	Certified Concentration ± associated uncertainty U, u=k*u (k=2)
Benzylfentanyl- ¹³ C ₆	1.000 ± 0.006 mg/mL

Metrological traceability: Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. See "Details on metrological traceability" on page 3.

Measurement method: The certified value is calculated from high precision weighing of thoroughly characterized starting material. See "Details about certification process" on page 3.

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.

Minimum sample size: 1 µL for quantitative applications

Instructions for handling and correct use: Concentration is corrected for chromatographic purity, residual water, residual solvents, and residual inorganics. No adjustment required before 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. Nominal concentration is adjusted for HCl content. No adjustment required before use.

For MS Applications, we advise laboratories not to mix lots during a single sequence.

Health and safety information: Danger. Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.

Accreditation: Cerilliant Corp. is accredited by the US accreditation authority ANAB as registered reference material producer AR-1353 in accordance with ISO 17034 and registered testing laboratory AT-1352 according to ISO/IEC 17025.




Darron Ellsworth, Quality Assurance Manager

August 23, 2021

Issue Date

Packaging:

2 mL amber USP Type 1 glass ampoule containing not less than 1 mL of certified solution. Ampoules are overfilled to ensure a minimum of 1 mL volume can be transferred when using a 1mL Class A volumetric pipette.

Details on starting materials:

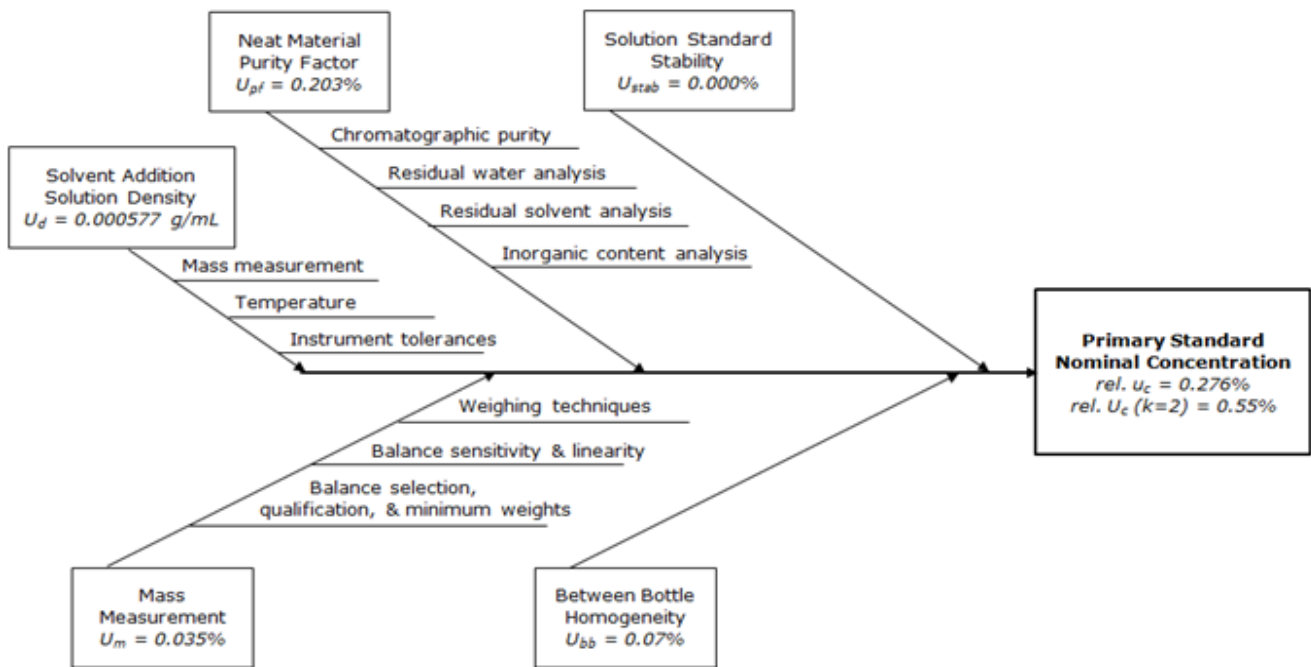
Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques and is assigned a Mass Balance Purity Factor. Spectral data is provided on subsequent pages of this CoA.

Certificate of Origin:

Cerilliant Corporation certifies no material of animal origin (BSE/TSE) was used in the preparation of this product. This material was manufactured in the USA.

Associated uncertainty:

The uncertainty has been calculated by statistical analysis of all aspects of our production system and incorporated uncertainty of the mass balance purity factor, material density, balance, weighing technique, and homogeneity. Uncertainty components of the gravimetrically prepared Primary Standard concentration are shown in the figure below. Uncertainty is expressed as an expanded uncertainty in accordance with ISO 17034 at the approximate 95% confidence interval using a coverage factor of k=2. Uncertainty contribution from neat material homogeneity was established to be negligible through establishment of process controls and verification of the control process. Stability uncertainty was determined to be negligible by regression analysis.



Details on metrological traceability:

- ♦ 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 0.10% relative error.
- ♦ Fill volume to predetermined specifications is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- ♦ The density and material Mass Balance Purity Factor of each raw material is traceable to the SI and higher order reference materials through mass measurement and instrument qualification and calibrations.

Details about certification process:

This standard has been prepared and certified under the ISO 17034, 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.

- ♦ Nominal concentration is calculated based on: the actual mass; Mass balance purity factor of the analyte(s); measured mass of the solution; and the density of the pure diluent at 20°C.
- ♦ 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.
- ♦ Additional certification information available upon request.

Solution Standard Verification

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

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

Standard Solution Assay Parameters		Calibration Curve	
Analysis Method:	HPLC/UV	Calibration Curve:	Linear Regression
Column:	Ascentis Express C18, 2.7 µm, 3.0 x 100 mm	Number of Points:	4
Mobile Phase:	Acetonitrile:0.1% Phosphoric acid in Water (30:70)	Linearity (r) :	1.000
Flow Rate:	1.5 mL/min		
Wavelength:	210 nm		
		Verified Concentration (mg/mL)	%RSD - Homogeneity
Standard Solution	Lot Number	Actual Results	Actual Results
New Lot	FN01211904	1.005	0.7
<ul style="list-style-type: none"> 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 Name:	Benzylfentanyl- ¹³ C ₆ HCl	Molecular Weight (base):	328.40
Material Lot:	FN12051801	Molecular Weight (salt):	364.86
Chemical Formula:	C ₁₅ ¹³ C ₆ H ₂₆ N ₂ O•HCl	Salt Adjustment:	1.111
CAS Number:	NA		

Material Characterization Summary

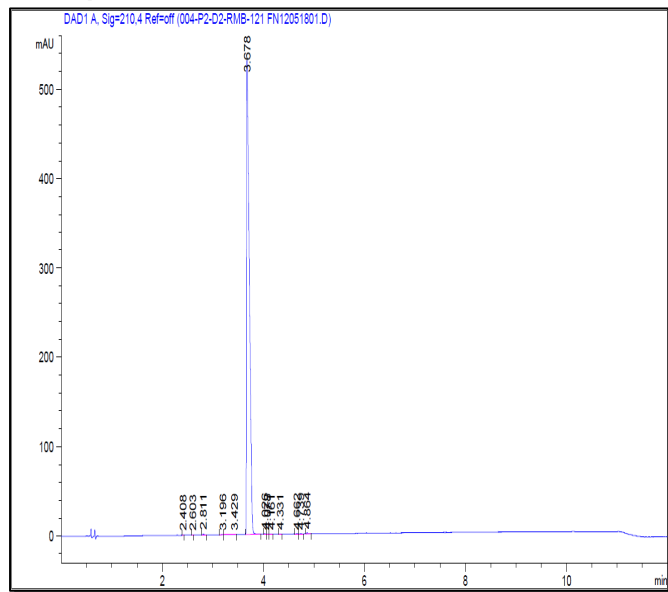
Analytical Test	Method	Results
Primary Chromatographic Purity by HPLC/UV Analysis	SP10-0102	99.6%
Secondary Chromatographic Purity by GC/FID Analysis	SP10-0101	99.6%
Identity by LC/MS Analysis	SP10-0107	Consistent with Structure
Isotopic Purity and Distribution by LC/MS SIM Analysis	SP10-0107	0.02% ¹³ C ₀ vs ¹³ C ₆
		0.02% ¹³ C ₀ to ¹³ C ₃ 2.31% ¹³ C ₅
		0.06% ¹³ C ₄ 97.54% ¹³ C ₆
Identity by ¹ H-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure
Residual Solvent Analysis by GC/FID Headspace	AM1087 ¹	None Detected
Residual Water Analysis by Karl Fischer Coulometry	AM1346 ¹	Below Quantitation Limit
Inorganic Content by Microash Analysis	SP10-0135	< 0.2%
Mass Balance Purity Factor		99.60%

¹ Validated analytical method

- ♦ 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 purity method was selected to optimize resolution of impurities while minimizing degradation of the analyte. Secondary purity methods with orthogonal detector capabilities from the primary purity method are used as controls to confirm an accurate purity value.
- ♦ 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.

Spectral and Physical Data

HPLC/UV



Column: Ascentis Express C18, 2.7 μ m, 3.0 x 100 mm

Mobile Phase: A: Acetonitrile
B: 0.1% Phosphoric acid in Water

Gradient:

Time (min)	% A	% B
0.0	10	90
8.0	70	30
10.0	70	30
10.1	10	90

Flow Rate: 0.7 mL/min

Wavelength: 210 nm

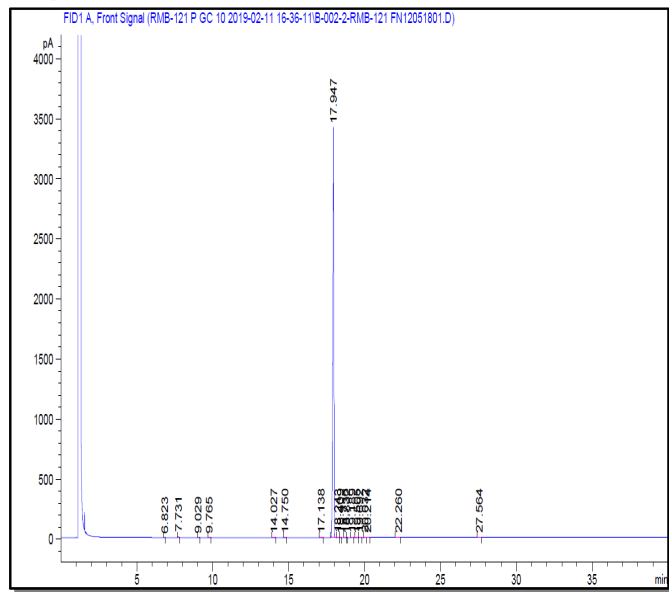
Sample Name: FN12051801

Acquired: February 08, 2019

Peak #	Ret Time	Area %
1	2.41	0.01
2	2.60	0.01
3	2.81	0.08
4	3.20	0.02
5	3.43	0.03
6	3.68	99.62
7	4.03	0.03
8	4.08	0.01
9	4.16	0.01
10	4.33	0.02
11	4.66	0.03
12	4.74	0.03
13	4.86	0.10

Spectral and Physical Data (cont.)

GC/FID

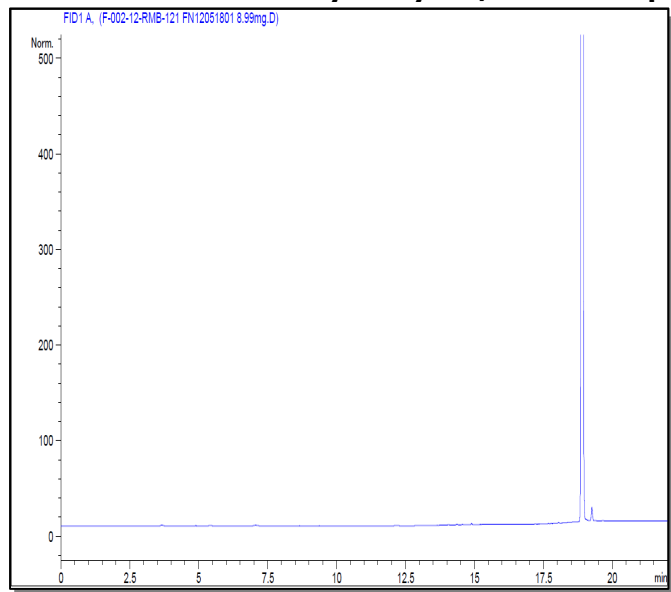


Column: DB-5ms, 30 m x 0.53 mm ID,
Temp Program: 40°C to 200°C at 40°C/min
 200°C to 300°C at 5°C/min hold 16 min
Injector Temp: Cool-on-Column
Detector Temp: 325°C
Sample Name: FN12051801
Acquired: February 11, 2019

Peak #	Ret Time	Area %
1	6.82	0.00
2	7.73	0.13
3	9.03	0.00
4	9.77	0.01
5	14.03	0.00
6	14.75	0.01
7	17.14	0.01
8	17.95	99.61
9	18.24	0.03
10	18.41	0.00
11	18.74	0.01
12	18.83	0.00
13	19.19	0.02
14	19.51	0.01
15	19.69	0.02
16	20.03	0.01
17	20.21	0.09
18	22.26	0.03
19	27.56	0.01

Spectral and Physical Data (cont.)

Residual Solvent Analysis by GC/FID Headspace



Column: DB-ALC1 30 m x 0.53 mm,
Temp Program: 40°C hold 12 min to 220°C at 40°C/min hold 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

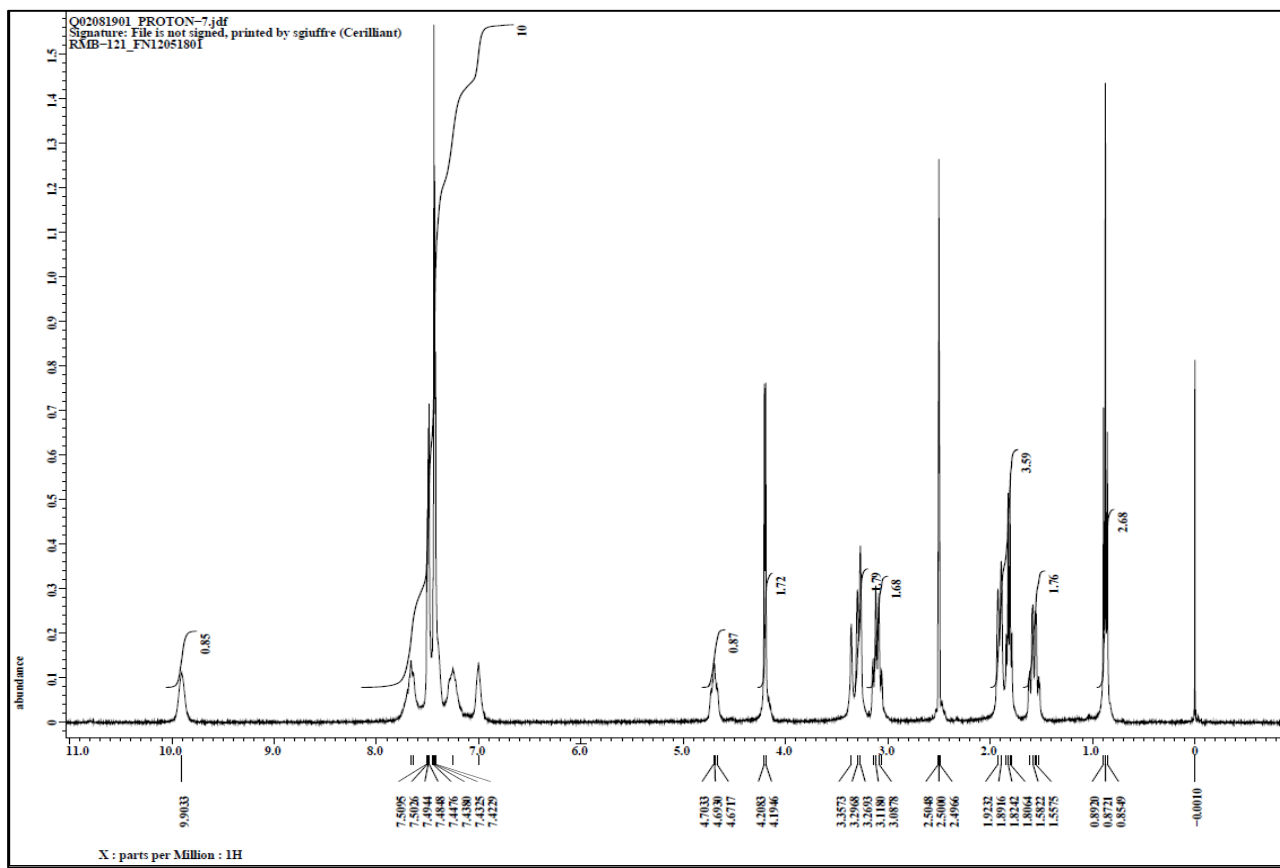
Sample Name: FN12051801
Acquired: February 14, 2019

Peak	Compound	Area	Weight %
1	NMP	NA	NA
Total			ND

ND - None Detected

¹H NMR

Instrument: JEOL ECS 400
Solvent: DMSO-D₆



Spectral and Physical Data (cont.)

LC/MS

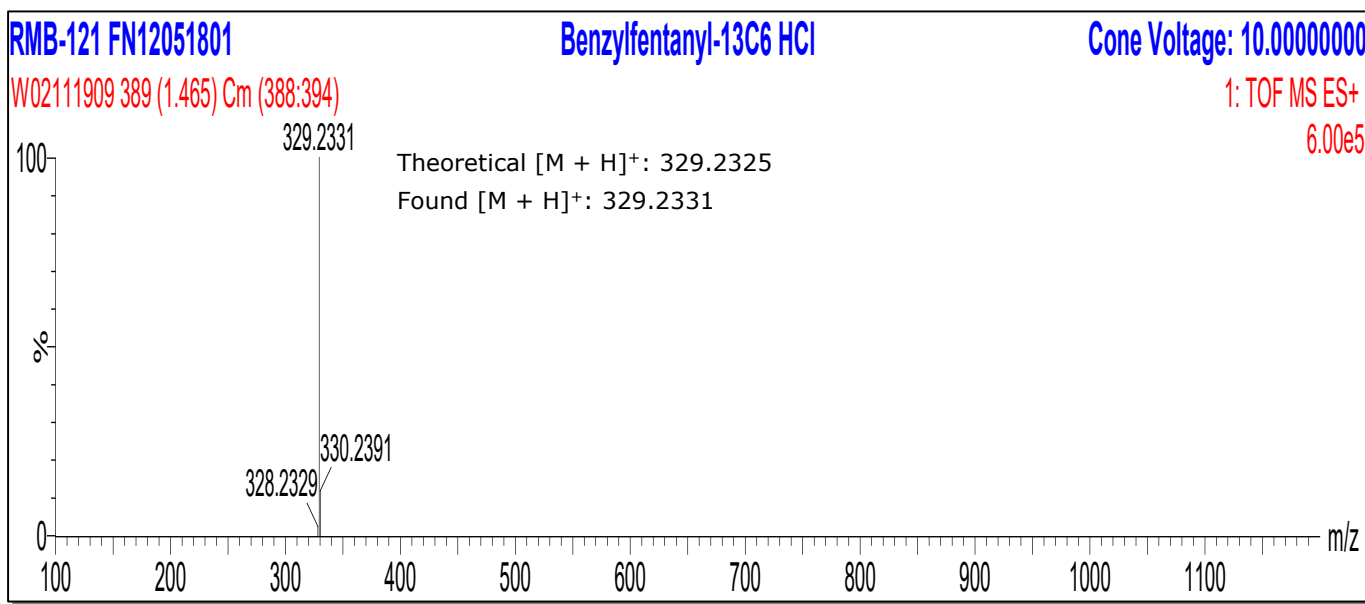
Column: Ascentis Express C18, 2.7 μ m,
3.0 x 50 mm

Mobile Phase: A: 0.1% Formic acid in Water
B: Acetonitrile

Gradient:

Time (min)	% A	% B
0.0	80	20
0.5	80	20
4.0	20	80
5.8	20	80
6.0	80	20
8.0	80	20

Flow Rate: 0.4 mL/min
Scan Range: 100-1200 amu
Ionization: Electrospray, Positive Ion
Instrument: Waters XEVO G2 QTOF
Acquired: February 11, 2019



Spectral and Physical Data (cont.)

Isotopic Purity by LC/MS

Column: Ascentis Express C18, 2.7 μ m,
3.0 x 50 mm

Mobile Phase: A: 0.1% Formic acid in Water
B: Acetonitrile

Gradient:

Time (min)	% A	% B
0.0	80	20
0.5	80	20
4.0	20	80
5.8	20	80
6.0	80	20
8.0	80	20

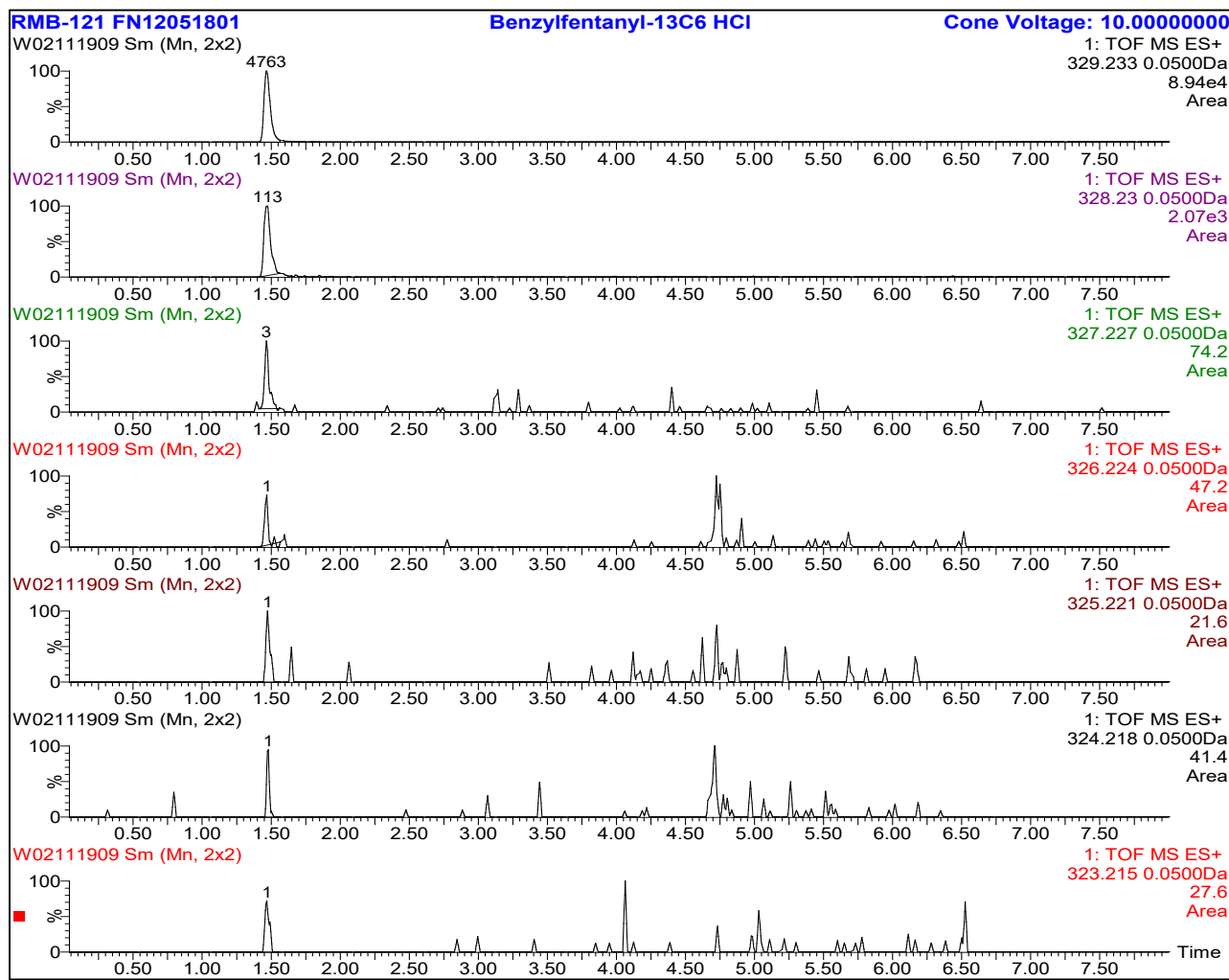
Flow Rate: 0.4 mL/min

Scan Range: 323-329 amu

Ionization: Electrospray, Positive Ion

Instrument: Waters XEVO G2 QTOF

Acquired: February 11, 2019



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 a related product (B-079-0.5ML, Benzylfentanyl HCl) 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.

Long Term Stability: Long term stability has been assessed for Freezer storage (-10 °C to -25 °C) conditions. Stability of a minimum of 30 months has been established through real-time stability studies.

Commutability

This standard is a solution of a pure substance in an organic solvent and is a Primary Standard. This Primary Standard is suitable for use in the preparation of calibrators and/or controls in any biological matrix. This standard is not in a biological matrix and therefore commutability to methods or standards in biological matrices does not apply.

COA Revision History

Revision No.	Date	Reason for Revision
00	March 01, 2019	Initial version.
01	February 19, 2020	Revised Retest Date from April 2020 to February 2021.
		Added Long Term Stability Section.
02	December 14, 2020	Revised Retest Date from February 2021 to November 2021.
03	August 23, 2021	Revised Retest Date from November 2021 to August 2022.

Certified Reference Material - Certificate of Analysis

Fentanyl, Primary Measurement Standard

N-Phenyl-N-[1-(2-phenylethyl)-4-piperidiny]propanamide

Product No.: F-013-1ML
Lot No.: FE12281801
Description of CRM: Fentanyl in Methanol (Solution)
Expiration Date: January 2024 See Section "Stability Assessment".
Storage: Store unopened in freezer (-10 °C to -25 °C).
Shipping: Ambient. See Section "Stability Assessment".
Chemical formula: C₂₂H₂₈N₂O
CAS No.: 437-38-7
Regulatory: USDEA Exempt | Canadian TK # 61-1188

Cerilliant Quality

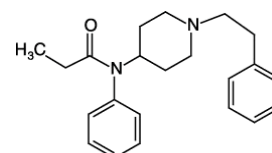
ISO 17034

ISO/IEC 17025

ISO 13485

ISO 14001

ISO 9001



Analyte	Certified Concentration ± associated uncertainty U, u=k*u (k=2)
Fentanyl	1.000 ± 0.006 mg/mL

Metrological traceability: Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. See "Details on metrological traceability" on page 2.

Measurement method: The certified value is calculated from high precision weighing of thoroughly characterized starting material. See "Details about certification process" on page 2.

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.

Minimum sample size: 1 µL for quantitative applications

Instructions for handling and correct use: Concentration is corrected for chromatographic purity, residual solvents and residual inorganics. No adjustment required before 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.

Health and safety information: Danger. Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.

Accreditation: Cerilliant Corp. is accredited by the US accreditation authority ANAB as registered reference material producer AR-1353 in accordance with ISO 17034 and registered testing laboratory AT-1352 according to ISO/IEC 17025.




Darron Ellsworth, Quality Assurance Manager

January 25, 2019

Issue Date

Packaging:

2 mL amber USP Type 1 glass ampoule containing not less than 1 mL of certified solution. Ampoules are overfilled to ensure a minimum of 1 mL volume can be transferred when using a 1mL Class A volumetric pipette.

Details on starting materials:

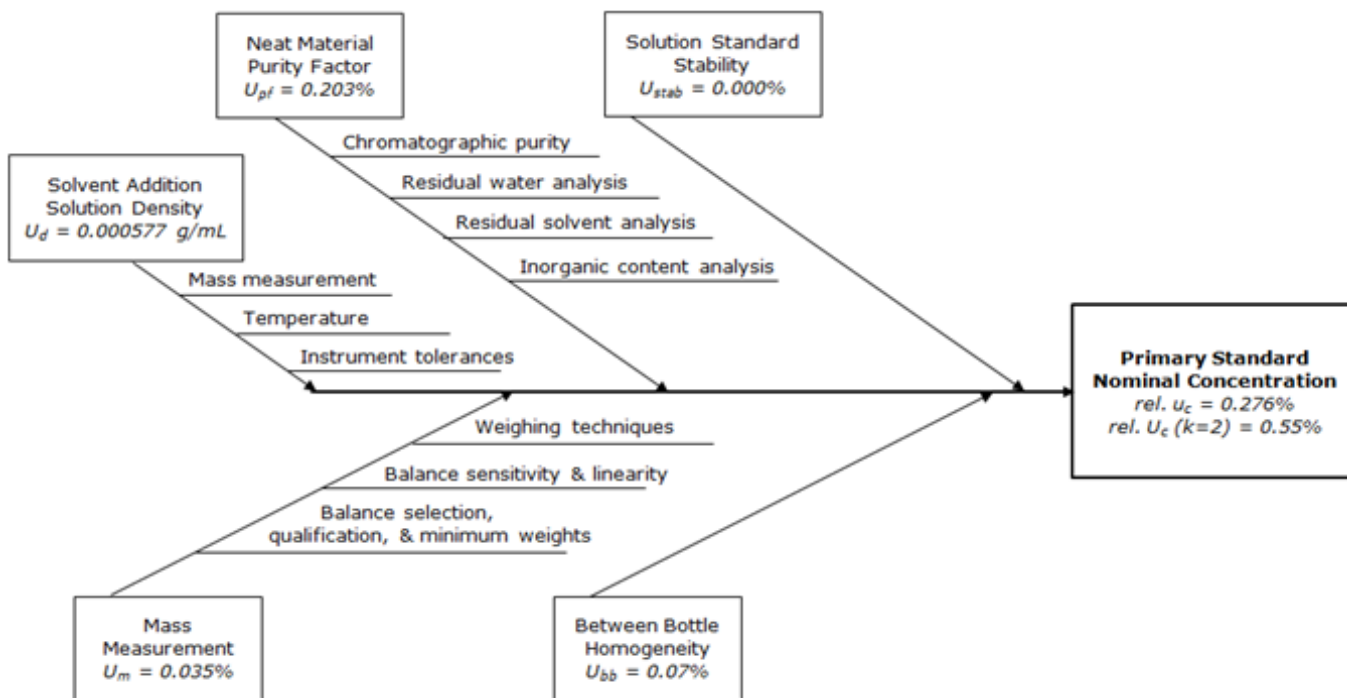
Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques and is assigned a Mass Balance Purity Factor. Spectral data is provided on subsequent pages of this CoA.

Certificate of Origin:

Cerilliant Corporation certifies no material of animal origin (BSE/TSE) was used in the preparation of this product. This material was manufactured in the USA.

Associated uncertainty:

The uncertainty has been calculated by statistical analysis of all aspects of our production system and incorporated uncertainty of the mass balance purity factor, material density, balance, weighing technique, and homogeneity. Uncertainty components of the gravimetrically prepared Primary Standard concentration are shown in the figure below. Uncertainty is expressed as an expanded uncertainty in accordance with ISO 17034 at the approximate 95% confidence interval using a coverage factor of $k=2$. Uncertainty contribution from neat material homogeneity was established to be negligible through establishment of process controls and verification of the control process. Stability uncertainty was determined to be negligible by regression analysis.



Details on metrological traceability:

- ♦ 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 0.10% relative error.
- ♦ Fill volume to predetermined specifications is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- ♦ The density and material Mass Balance Purity Factor of each raw material is traceable to the SI and higher order reference materials through mass measurement and instrument qualification and calibrations.

Details about certification process:

This standard has been prepared and certified under the ISO 17034, 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.

- ♦ Nominal concentration is calculated based on: the actual mass; Mass balance purity factor of the analyte(s); measured mass of the solution; and the density of the pure diluent at 20°C.
- ♦ 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.
- ♦ Additional certification information available upon request.

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 Measurement Standard have been fulfilled and validated under ISO 13485.

Standard Solution Assay Parameters		Calibration Curve	
Analysis Method:	HPLC/UV	Calibration Curve:	Linear Regression
Column:	Ascentis Express C18, 2.7 µm, 3.0 x 100 mm	Number of Points:	4
Mobile Phase:	Acetonitrile:0.1% Phosphoric acid in Water (28:72)	Linearity (r) :	1.000
Flow Rate:	1.2 mL/min		
Wavelength:	210 nm		
		Verified Concentration (mg/mL)	%RSD - Homogeneity
Standard Solution	Lot Number	Actual Results	Actual Results
New Lot	FE12281801	0.993	0.8
Previous Lot	FE06151802	1.002	0.3
<ul style="list-style-type: none"> ♦ 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. 			

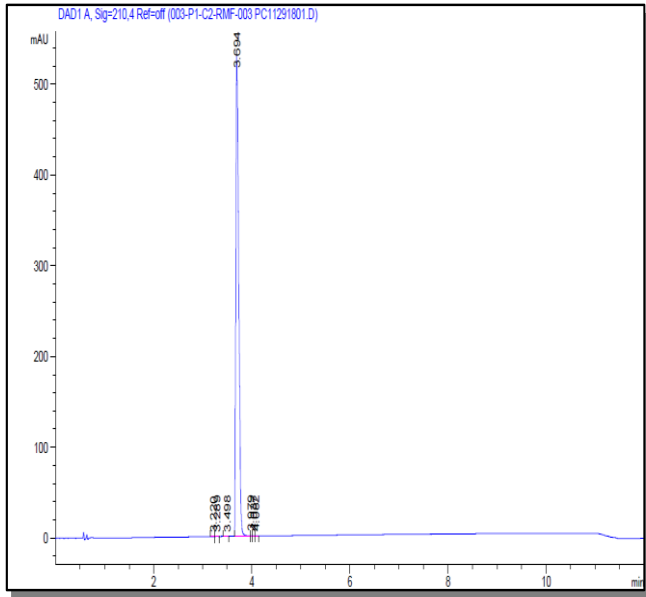
Analyte Certification - Mass Balance Purity Factor

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 is utilized to calculate the weighing adjustment necessary to ensure accuracy of the solution standard concentration.

Material Name:	Fentanyl	Chemical Formula:	C ₂₂ H ₂₈ N ₂ O
Material Lot:	PC11291801	CAS Number:	437-38-7
		Molecular Weight:	336.47
Material Characterization Summary			
Analytical Test	Method	Results	
Primary Chromatographic Purity by HPLC/UV Analysis	SP10-0102	99.9%	
Secondary Chromatographic Purity by GC/FID Analysis	SP10-0101	99.9%	
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	None Detected	
Residual Water Analysis by Karl Fischer Coulometry	AM1346 ¹	Not Detected	
Inorganic Content by Microash Analysis	SP10-0135	< 0.2%	
Mass Balance Purity Factor		99.90%	
¹ Validated analytical method			
♦ 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 purity method was selected to optimize resolution of impurities while minimizing degradation of the analyte. Secondary purity methods with orthogonal detector capabilities from the primary purity method are used as controls to confirm an accurate purity value.			
♦ 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.			

Spectral and Physical Data

HPLC/UV



Column: Ascentis Express C18, 2.7 μ m, 3.0 x 100 mm

Mobile Phase: A: Acetonitrile
B: 0.1% Phosphoric acid in Water

Gradient:

Time (min)	% A	% B
0.0	10	90
8.0	70	30
10.0	70	30
10.1	10	90

Flow Rate: 0.7 mL/min

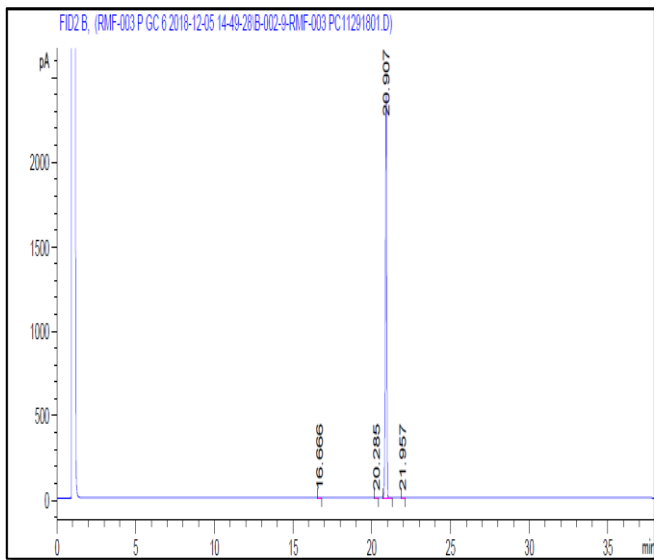
Wavelength: 210 nm

Sample Name: PC11291801

Acquired: December 06, 2018

Peak #	Ret Time	Area %
1	3.22	0.01
2	3.29	0.05
3	3.50	0.01
4	3.69	99.88
5	3.98	0.02
6	4.04	0.02
7	4.08	0.01

GC/FID



Column: DB-35ms, 30 m x 0.53 mm ID, 1.0 μ 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

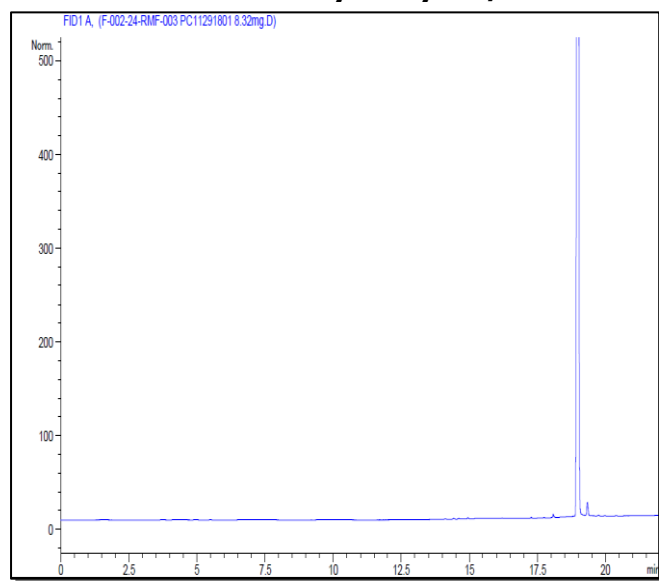
Sample Name: PC11291801

Acquired: December 05, 2018

Peak #	Ret Time	Area %
1	16.67	0.01
2	20.29	0.05
3	20.91	99.94
4	21.96	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 hold 12 min to 220°C at 40°C/min hold 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

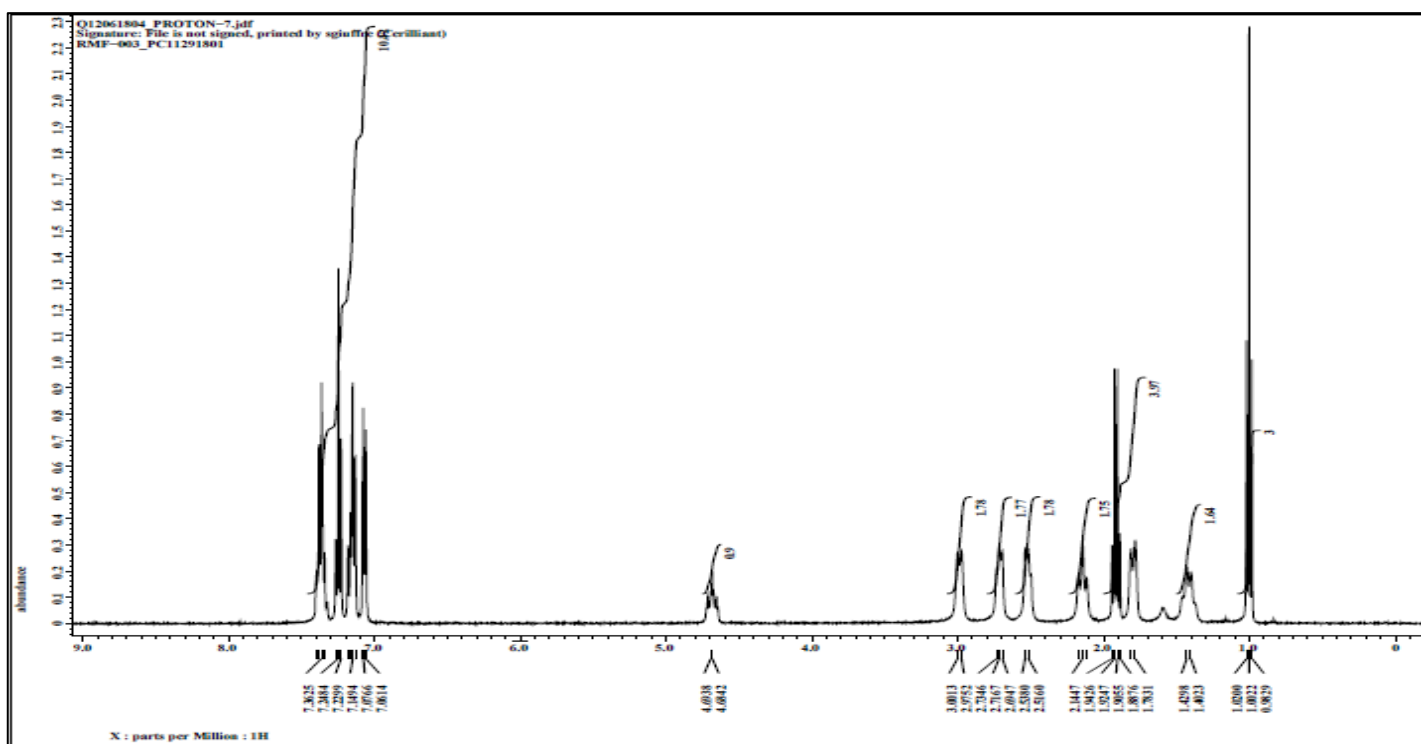
Sample Name: PC11291801
Acquired: December 06, 2018

Peak	Compound	Area	Weight %
1	NMP	NA	NA
Total			ND

ND - None Detected

¹H NMR

Instrument: JEOL ECS 400
Solvent: Chloroform-D



Spectral and Physical Data (cont.)

LC/MS

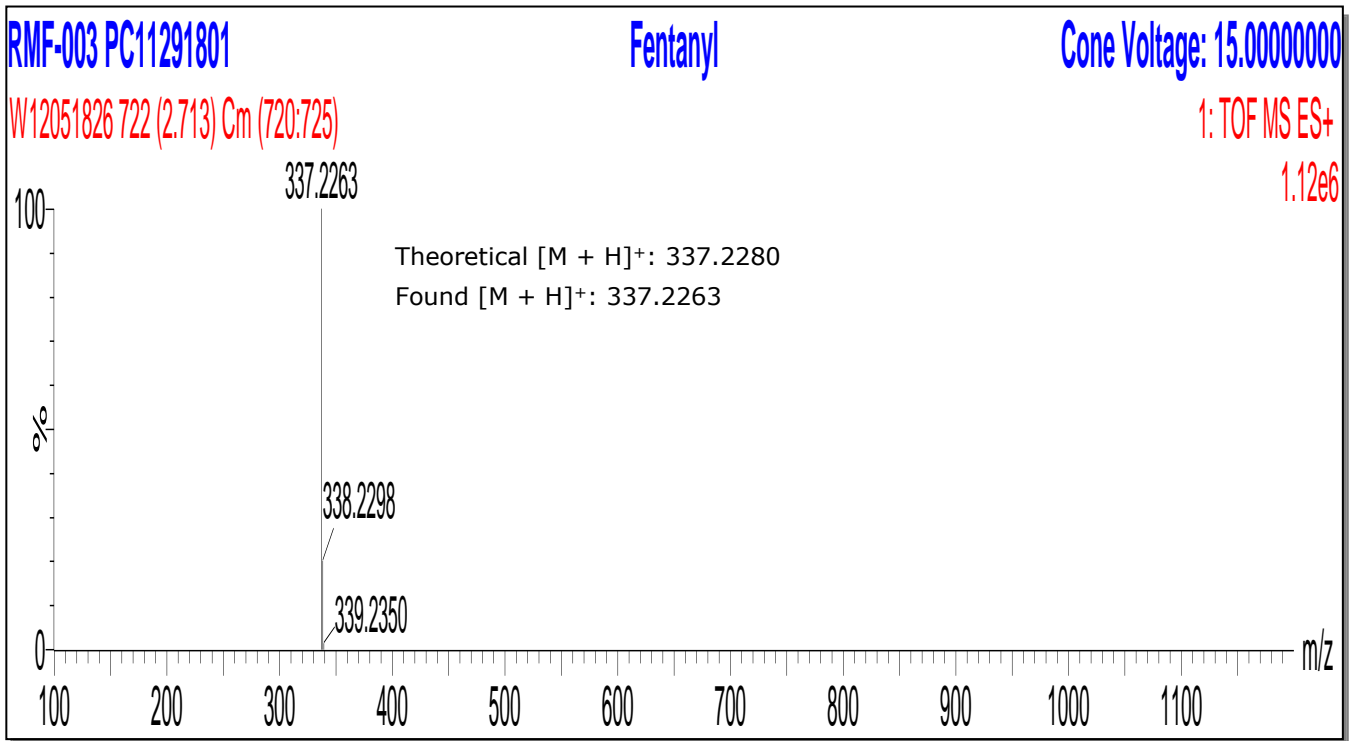
Column: Ascentis Express C18, 2.7 μ m, 3.0 x 50 mm

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
Instrument: Waters XEVO G2 QTOF
Acquired: December 05, 2018



Stability		
<i>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.</i>		
Short Term Stability: <i>A summary of accelerated stability findings for this product is listed below.</i>		
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: <i>Stability studies support the transport of this product at ambient conditions.</i>		
Long Term Stability: <i>Long term stability has been assessed for Freezer storage (-10 °C to -25 °C) conditions. Stability of a minimum of 60 months has been established through real-time stability studies.</i>		

Commutability

This standard is a solution of a pure substance in an organic solvent and is a Primary Standard. This Primary Standard is suitable for use in the preparation of calibrators and/or controls in any biological matrix. This standard is not in a biological matrix and therefore commutability to methods or standards in biological matrices does not apply.

COA Revision History

Revision No.	Date	Reason for Revision
00	January 25, 2019	Initial version.

Certified Reference Material - Certificate of Analysis

U-47700, Primary Measurement Standard (±)-trans-3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methyl-benzamide

Cerilliant Quality

ISO 17034

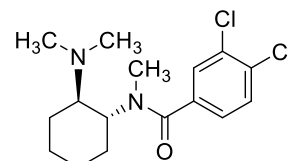
ISO/IEC 17025

ISO 13485

ISO 14001

ISO 9001

Product No.: U-003-1ML
Lot No.: FE12241802
Description of CRM: U-47700 in Methanol (Solution)
Expiration: February 2022 See Section "Stability Assessment".
Storage: Store unopened in freezer (-10 °C to -25 °C).
Shipping: Ship cold. See Section "Stability Assessment".
Chemical formula: C₁₆H₂₂Cl₂N₂O
CAS No.: 82657-23-6
Regulatory: USDEA Exempt | Canadian TK # 61-1674



racemic *trans*

Analyte	Certified Concentration ± associated uncertainty U, u=k*u (k=2)
U-47700	1.000 ± 0.006 mg/mL

Metrological traceability: Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. See "Details on metrological traceability" on page 2.

Measurement method: The certified value is calculated from high precision weighing of thoroughly characterized starting material. See "Details about certification process" on page 2.

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.

Minimum sample size: 1 µL for quantitative applications

Instructions for handling and correct use: Concentration is corrected for chromatographic purity, residual solvents and residual inorganics. No adjustment required before 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.

Health and safety information: Danger. Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.

Accreditation: Cerilliant Corp. is accredited by the US accreditation authority ANAB as registered reference material producer AR-1353 in accordance with ISO 17034 and registered testing laboratory AT-1352 according to ISO/IEC 17025.




Darron Ellsworth, Quality Assurance Manager

December 30, 2020

Issue Date

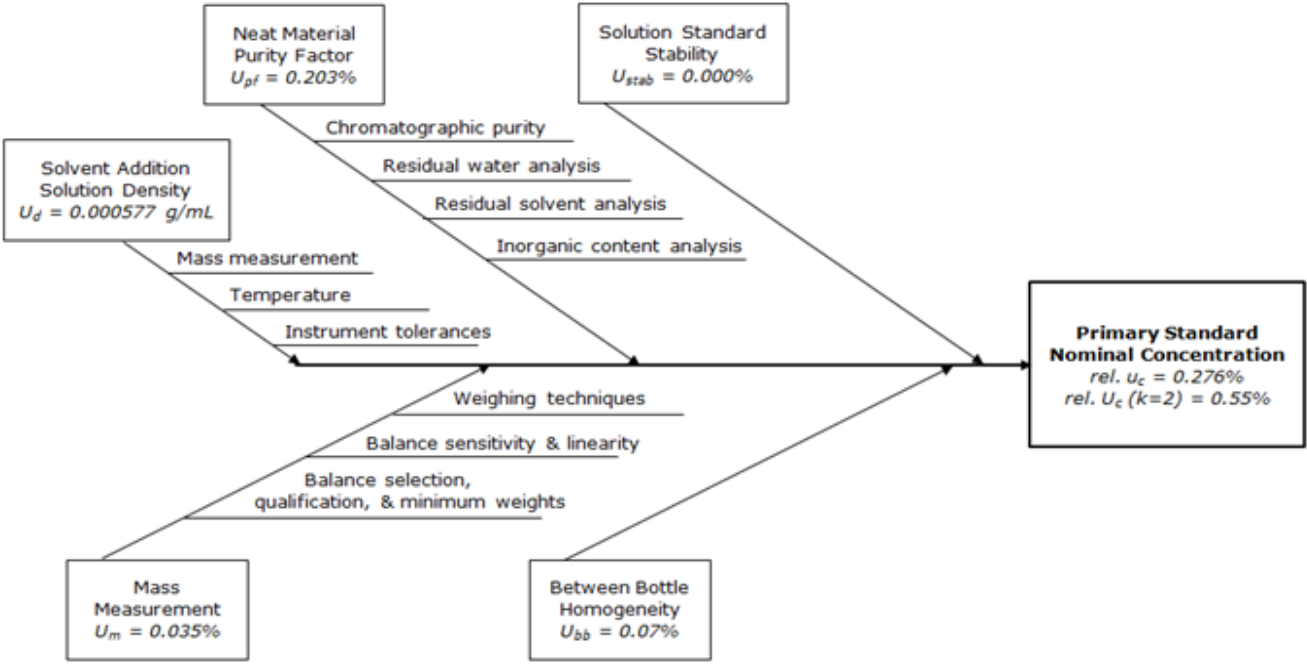
Packaging: 2 mL amber USP Type 1 glass ampoule containing not less than 1 mL of certified solution. Ampoules are overfilled to ensure a minimum of 1 mL volume can be transferred when using a 1mL Class A volumetric pipette.

Details on starting materials: Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques and is assigned a Mass Balance Purity Factor. Spectral data is provided on subsequent pages of this CoA.

Certificate of Origin: Cerilliant Corporation certifies no material of animal origin (BSE/TSE) was used in the preparation of this product. This material was manufactured in the USA.

Associated uncertainty:

The uncertainty has been calculated by statistical analysis of all aspects of our production system and incorporated uncertainty of the mass balance purity factor, material density, balance, weighing technique, and homogeneity. Uncertainty components of the gravimetrically prepared Primary Standard concentration are shown in the figure below. Uncertainty is expressed as an expanded uncertainty in accordance with ISO 17034 at the approximate 95% confidence interval using a coverage factor of k=2. Uncertainty contribution from neat material homogeneity was established to be negligible through establishment of process controls and verification of the control process. Stability uncertainty was determined to be negligible by regression analysis.



Details on metrological traceability:

- ♦ 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 0.10% relative error.
- ♦ Fill volume to predetermined specifications is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- ♦ The density and material Mass Balance Purity Factor of each raw material is traceable to the SI and higher order reference materials through mass measurement and instrument qualification and calibrations.

Details about certification process:

This standard has been prepared and certified under the ISO 17034, 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.

- ♦ Nominal concentration is calculated based on: the actual mass; Mass balance purity factor of the analyte(s); measured mass of the solution; and the density of the pure diluent at 20°C.
- ♦ 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.
- ♦ Additional certification information available upon request.

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 Measurement Standard have been fulfilled and validated under ISO 13485.

Standard Solution Assay Parameters		Calibration Curve	
Analysis Method:	HPLC/UV	Calibration Curve:	Linear Regression
Column:	Ascentis Express Phenyl-Hexyl, 2.7 µm, 3.0 x 100 mm	Number of Points:	4
Mobile Phase:	Acetonitrile:0.1% Phosphoric acid in Water (35:65)	Linearity (r):	1.000
Flow Rate:	1.5 mL/min		
Wavelength:	220 nm		
		Verified Concentration (mg/mL)	%RSD - Homogeneity
Standard Solution	Lot Number	Actual Results	Actual Results
New Lot	FE12241802	1.004	0.2
Previous Lot	FE03201801	1.018	0.6
<ul style="list-style-type: none"> ♦ 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 is utilized to calculate the weighing adjustment necessary to ensure accuracy of the solution standard concentration.

Material Name:	U-47700	Chemical Formula:	C ₁₆ H ₂₂ Cl ₂ N ₂ O
Material Lot:	FC09271805	CAS Number:	82657-23-6
		Molecular Weight:	329.26

Material Characterization Summary

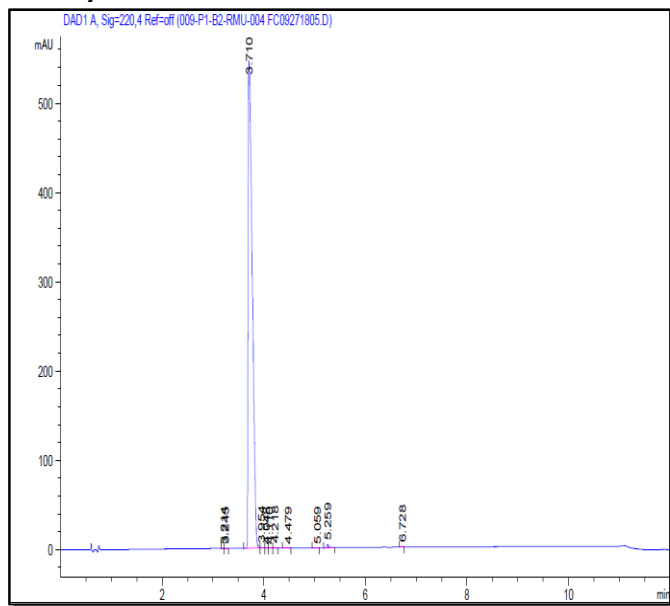
Analytical Test	Method	Results
Primary Chromatographic Purity by HPLC/UV Analysis	SP10-0102	99.5%
Secondary Chromatographic Purity by GC/FID Analysis	SP10-0101	99.5%
Identity by LC/MS Analysis	SP10-0107	Consistent with Structure
Identity by ¹ H-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure
Residual Solvent Analysis by GC/FID Headspace	AM1087 ¹	None Detected
Residual Water Analysis by Karl Fischer Coulometry	AM1346 ¹	Not Detected
Inorganic Content by Microash Analysis	SP10-0135	< 0.2%
Mass Balance Purity Factor		99.52%

¹ Validated analytical method.

- ♦ 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 purity method was selected to optimize resolution of impurities while minimizing degradation of the analyte. Secondary purity methods with orthogonal detector capabilities from the primary purity method are used as controls to confirm an accurate purity value.
- ♦ 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.

Spectral and Physical Data

HPLC/UV



Column: Ascentis Express Phenyl-Hexyl,
2.7 μ m, 3.0 x 100 mm

Mobile Phase: A: Acetonitrile
B: 0.1% Phosphoric acid in Water

Gradient:

Time (min)	% A	% B
0.0	10	90
8.0	80	20
10.0	80	20
10.1	10	90

Flow Rate: 0.7 mL/min

Wavelength: 220 nm

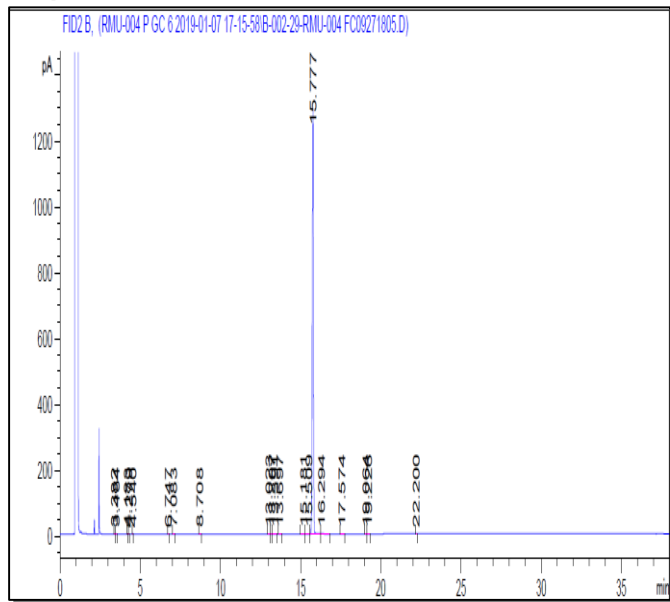
Sample Name: FC09271805

Acquired: January 07, 2019

Peak #	Ret Time	Area %
1	3.21	0.01
2	3.25	0.01
3	3.71	99.56
4	3.95	0.06
5	4.05	0.02
6	4.11	0.02
7	4.22	0.02
8	4.48	0.01
9	5.06	0.01
10	5.26	0.25
11	6.73	0.03

Spectral and Physical Data (cont.)

GC/FID

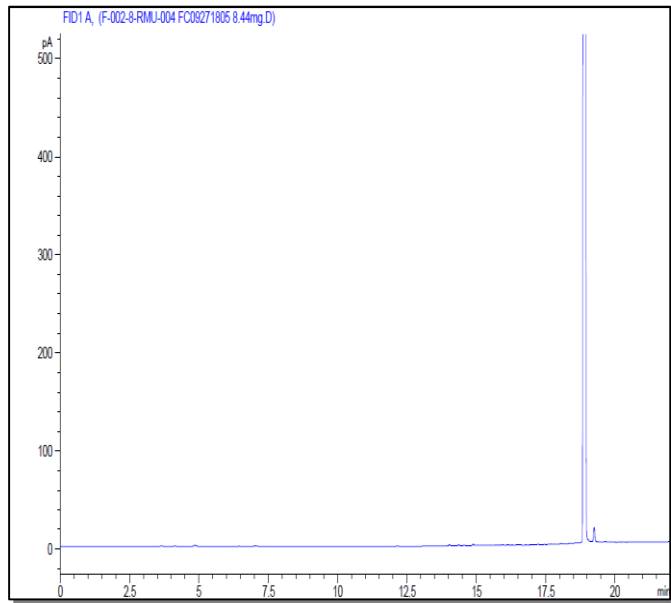


Column: DB-35ms, 30 m x 0.53 mm ID, 1.0 µm film thickness
Temp Program: 40°C to 200°C at 40°C/min
 200°C to 280°C at 5°C/min hold 18 min
Injector Temp: Cool-on-Column
Detector Temp: 325°C
Sample Name: FC09271805
Acquired: January 07, 2019

Peak #	Ret Time	Area %
1	3.38	0.03
2	3.48	0.07
3	4.18	0.00
4	4.28	0.00
5	4.54	0.04
6	6.75	0.01
7	7.08	0.00
8	8.71	0.01
9	13.02	0.01
10	13.21	0.00
11	13.39	0.02
12	13.70	0.03
13	15.18	0.01
14	15.51	0.02
15	15.78	99.51
16	16.29	0.21
17	17.57	0.02
18	19.06	0.00
19	19.23	0.01
20	22.20	0.00

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

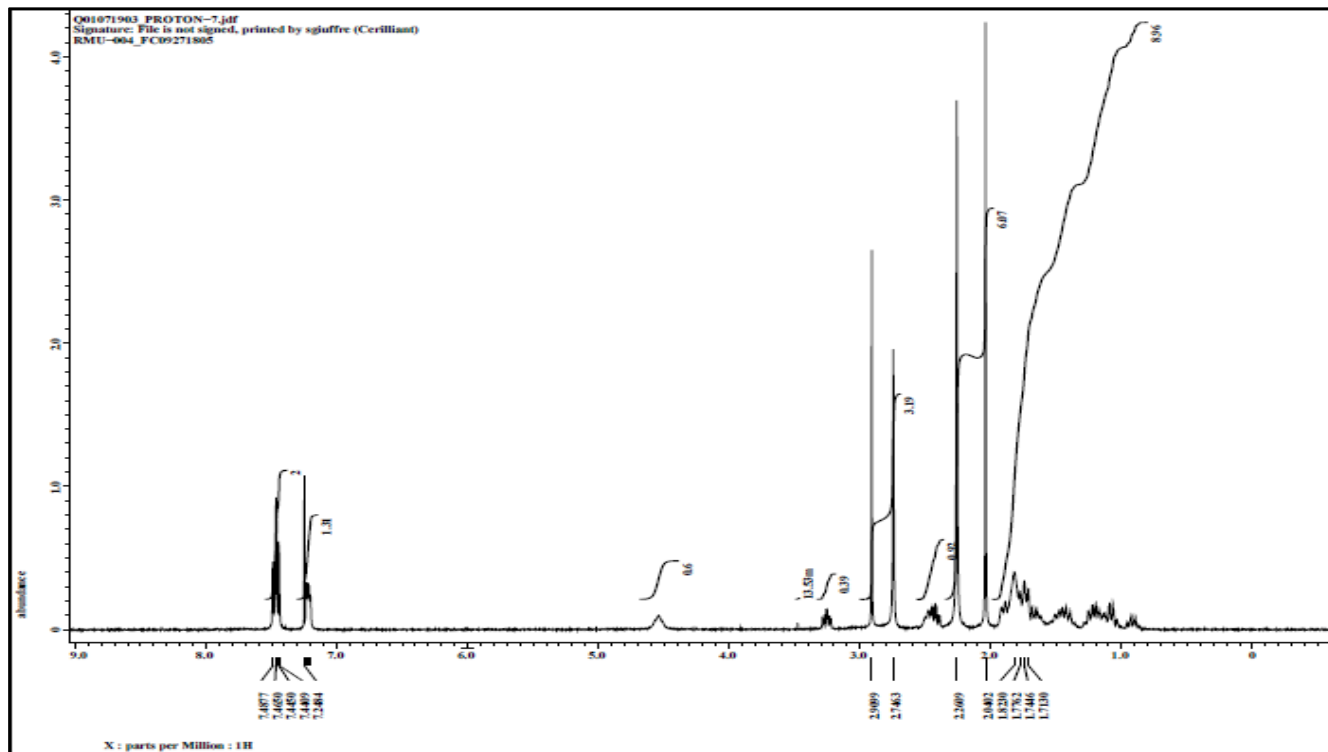
Sample Name: FC09271805
Acquired: September 02, 2018

Peak	Compound	Area	Weight %
1	NMP	NA	NA
Total			ND

ND - None Detected

¹H NMR

Instrument: JEOL ECS 400
Solvent: Chloroform-D



Spectral and Physical Data (cont.)

LC/MS

Column: Ascentis Express C18, 2.7 μ m, 3.0 x 50 mm

Mobile Phase: A: 0.1% Formic acid in Water

B: Acetonitrile

Gradient:

Time (min)	% A	% B
0.0	80	20
0.5	80	20
4.0	20	80
5.8	20	80
6.0	80	20
8.0	80	20

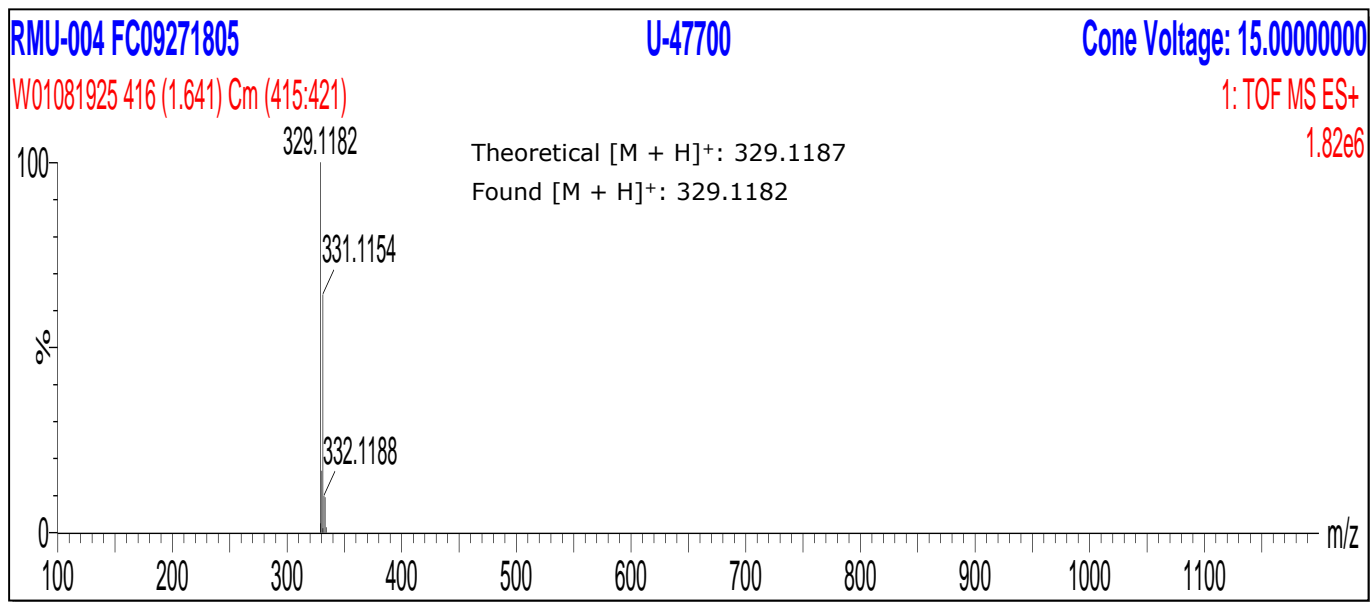
Flow Rate: 0.4 mL/min

Scan Range: 100-1200 amu

Ionization: Electrospray, Positive Ion

Instrument: Waters XEVO G2 QTOF

Acquired: January 08, 2019



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	1.58% decrease in purity was noted after two weeks.

Transport/Shipping: Ship cold.

Long Term Stability: Long term stability has been assessed for Freezer storage (-10 °C to -25 °C) conditions. Stability of a minimum of 37 months has been established through real-time stability studies.

Commutability

This standard is a solution of a pure substance in an organic solvent and is a Primary Standard. This Primary Standard is suitable for use in the preparation of calibrators and/or controls in any biological matrix. This standard is not in a biological matrix and therefore commutability to methods or standards in biological matrices does not apply.

COA Revision History

Revision No.	Date	Reason for Revision
00	February 25, 2019	Initial version.
01	December 30, 2020	Revised Retest Date of May 2021 to Expiration of February 2022.

Certified Reference Material - Certificate of Analysis

U-49900, Primary Measurement Standard

(±)-trans-3,4-Dichloro-N-[2-(diethylamino)cyclohexyl]-N-methylbenzamide

Product No.: U-009-1ML
Lot No.: FN01161901
Description of CRM: U-49900 in Methanol (Solution)
Retest Date: October 2021 See Section "Stability Assessment".
Storage: Store unopened in freezer (-10 °C to -25 °C).
Shipping: Ship cold. See Section "Stability Assessment".
Chemical formula: C₁₈H₂₆Cl₂N₂O
CAS No.: 67579-76-4
Regulatory: Canadian TK # 61-1704

Cerilliant Quality

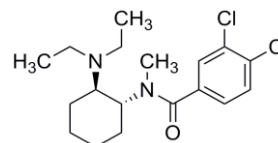
ISO 17034

ISO/IEC 17025

ISO 13485

ISO 14001

ISO 9001



Analyte	Certified Concentration ± associated uncertainty U, u=k*u (k=2)
U-49900	1.000 ± 0.006 mg/mL

Metrological traceability: Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. See "Details on metrological traceability" on page 2.

Measurement method: The certified value is calculated from high precision weighing of thoroughly characterized starting material. See "Details about certification process" on page 2.

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.

Minimum sample size: 1 µL for quantitative applications

Instructions for handling and correct use: Concentration is corrected for chromatographic purity, residual solvents and residual inorganics. No adjustment required before 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.

Health and safety information: Danger. Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.

Accreditation: Cerilliant Corp. is accredited by the US accreditation authority ANAB as registered reference material producer AR-1353 in accordance with ISO 17034 and registered testing laboratory AT-1352 according to ISO/IEC 17025.




Darron Ellsworth, Quality Assurance Manager

November 13, 2020

Issue Date

Packaging:

2 mL amber USP Type 1 glass ampoule containing not less than 1 mL of certified solution. Ampoules are overfilled to ensure a minimum of 1 mL volume can be transferred when using a 1mL Class A volumetric pipette.

Details on starting materials:

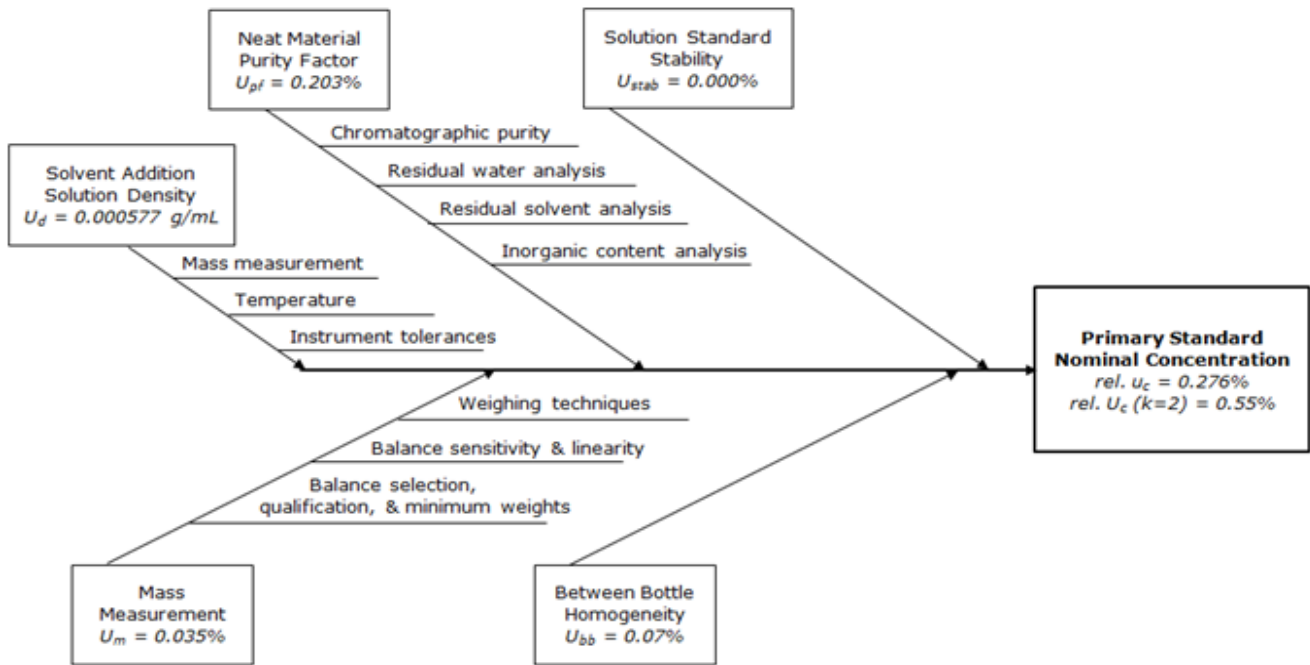
Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques and is assigned a Mass Balance Purity Factor. Spectral data is provided on subsequent pages of this CoA.

Certificate of Origin:

Cerilliant Corporation certifies no material of animal origin (BSE/TSE) was used in the preparation of this product. This material was manufactured in the USA.

Associated uncertainty:

The uncertainty has been calculated by statistical analysis of all aspects of our production system and incorporated uncertainty of the mass balance purity factor, material density, balance, weighing technique, and homogeneity. Uncertainty components of the gravimetrically prepared Primary Standard concentration are shown in the figure below. Uncertainty is expressed as an expanded uncertainty in accordance with ISO 17034 at the approximate 95% confidence interval using a coverage factor of k=2. Uncertainty contribution from neat material homogeneity was established to be negligible through establishment of process controls and verification of the control process. Stability uncertainty was determined to be negligible by regression analysis.



Details on metrological traceability:

- ♦ 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 0.10% relative error.
- ♦ Fill volume to predetermined specifications is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- ♦ The density and material Mass Balance Purity Factor of each raw material is traceable to the SI and higher order reference materials through mass measurement and instrument qualification and calibrations.

Details about certification process:

This standard has been prepared and certified under the ISO 17034, 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.

- ♦ Nominal concentration is calculated based on: the actual mass; Mass balance purity factor of the analyte(s); measured mass of the solution; and the density of the pure diluent at 20°C.
- ♦ 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.
- ♦ Additional certification information available upon request.

Solution Standard Verification

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

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

Standard Solution Assay Parameters		Calibration Curve	
Analysis Method:	HPLC/UV	Calibration Curve:	Linear Regression
Column:	Ascentis Express C18, 2.7 µm, 3.0 x 100 mm	Number of Points:	4
Mobile Phase:	Acetonitrile:0.1% Phosphoric acid in Water (35:65)	Linearity (r) :	1.000
Flow Rate:	1.5 mL/min		
Wavelength:	220 nm		
		Verified Concentration (mg/mL)	%RSD - Homogeneity
Standard Solution	Lot Number	Actual Results	Actual Results
New Lot	FN01161901	1.000	0.2
<ul style="list-style-type: none"> ♦ 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 is utilized to calculate the weighing adjustment necessary to ensure accuracy of the solution standard concentration.

Material Name:	U-49900	Chemical Formula:	C ₁₈ H ₂₆ Cl ₂ N ₂ O
Material Lot:	FN06281801	CAS Number:	67579-76-4
		Molecular Weight:	357.32

Material Characterization Summary

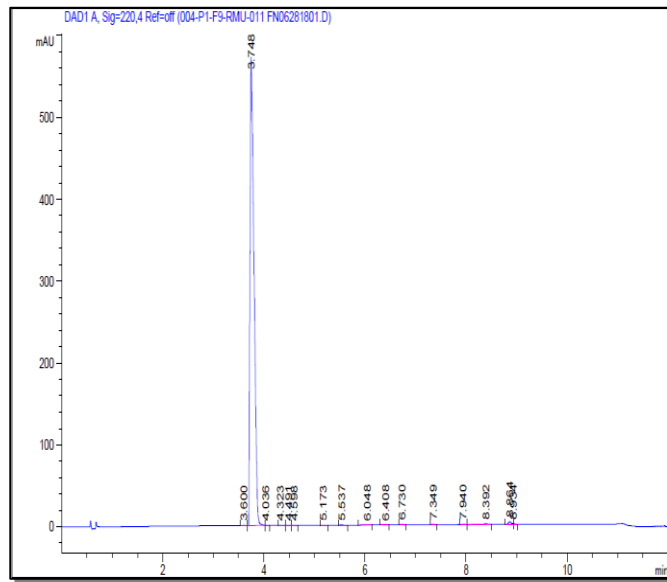
Analytical Test	Method	Results
Primary Chromatographic Purity by HPLC/UV Analysis	SP10-0102	99.0%
Secondary Chromatographic Purity by GC/FID Analysis	SP10-0101	99.7%
Identity by LC/MS Analysis	SP10-0107	Consistent with Structure
Identity by ¹ H-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure
Residual Solvent Analysis by GC/FID Headspace	AM1087 ¹	Below Quantitation Limit
Residual Water Analysis by Karl Fischer Coulometry	AM1346 ¹	None Detected
Inorganic Content by Microash Analysis	SP10-0135	< 0.2%
Mass Balance Purity Factor		99.02%

¹ Validated analytical method

- ♦ 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 purity method was selected to optimize resolution of impurities while minimizing degradation of the analyte. Secondary purity methods with orthogonal detector capabilities from the primary purity method are used as controls to confirm an accurate purity value.
- ♦ 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.

Spectral and Physical Data

HPLC/UV



Column: Ascentis Express C18, 2.7 μ m, 3.0 x 100 mm

Mobile Phase: A: Acetonitrile
B: 0.1% Phosphoric acid in Water

Gradient:

Time (min)	% A	% B
0.0	10	90
8.0	80	20
10.0	80	20
10.1	10	90

Flow Rate: 0.7 mL/min

Wavelength: 220 nm

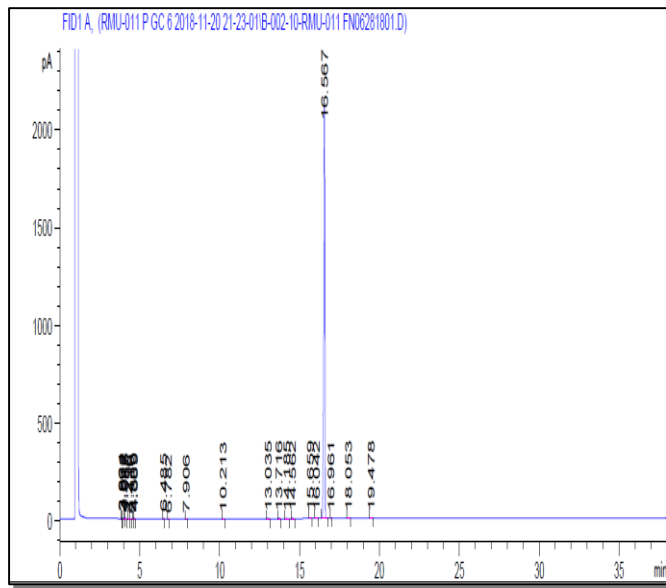
Sample Name: FN06281801

Acquired: November 29, 2018

Peak #	Ret Time	Area %
1	3.60	0.05
2	3.75	99.03
3	4.04	0.02
4	4.32	0.01
5	4.49	0.04
6	4.60	0.01
7	5.17	0.02
8	5.54	0.04
9	6.05	0.06
10	6.41	0.02
11	6.73	0.06
12	7.35	0.04
13	7.94	0.02
14	8.39	0.12
15	8.86	0.41
16	8.93	0.05

Spectral and Physical Data (cont.)

GC/FID



Column: DB-5ms, 30 m x 0.53 mm ID, 1.5 µm 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

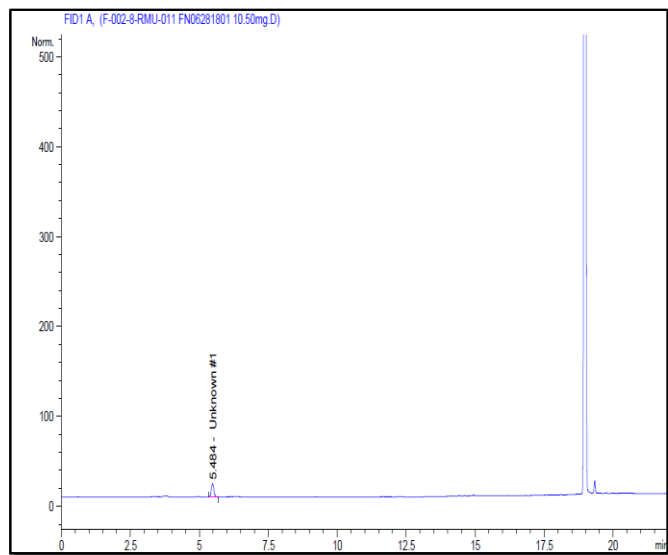
Sample Name: FN06281801

Acquired: November 20, 2018

Peak #	Ret Time	Area %
1	3.86	0.00
2	3.92	0.00
3	4.02	0.04
4	4.09	0.01
5	4.30	0.00
6	4.42	0.00
7	4.59	0.03
8	4.67	0.01
9	6.49	0.01
10	6.78	0.01
11	7.91	0.00
12	10.21	0.01
13	13.04	0.02
14	13.72	0.03
15	14.19	0.02
16	14.56	0.03
17	15.66	0.00
18	16.04	0.02
19	16.57	99.72
20	16.96	0.01
21	18.05	0.01
22	19.48	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 hold 12 min to 220°C at 40°C/min hold 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

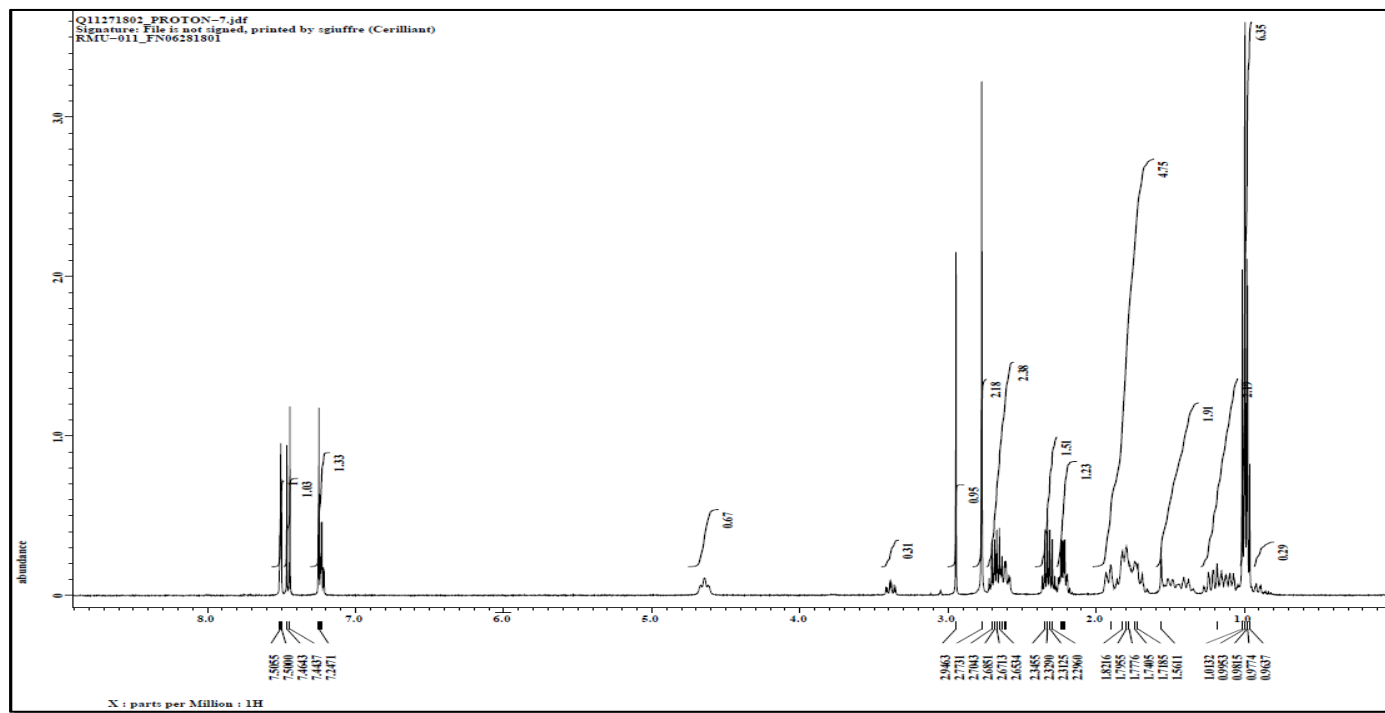
Sample Name: FN06281801
Acquired: November 20, 2018

Peak	Compound	Area	Weight %
1	Unknown #1	95.64	BQL
2	NMP	NA	NA
Total			BQL

BQL - Below Quantitation Limit

¹H NMR

Instrument: JEOL ECS 400
Solvent: Chloroform-D



Spectral and Physical Data (cont.)

LC/MS

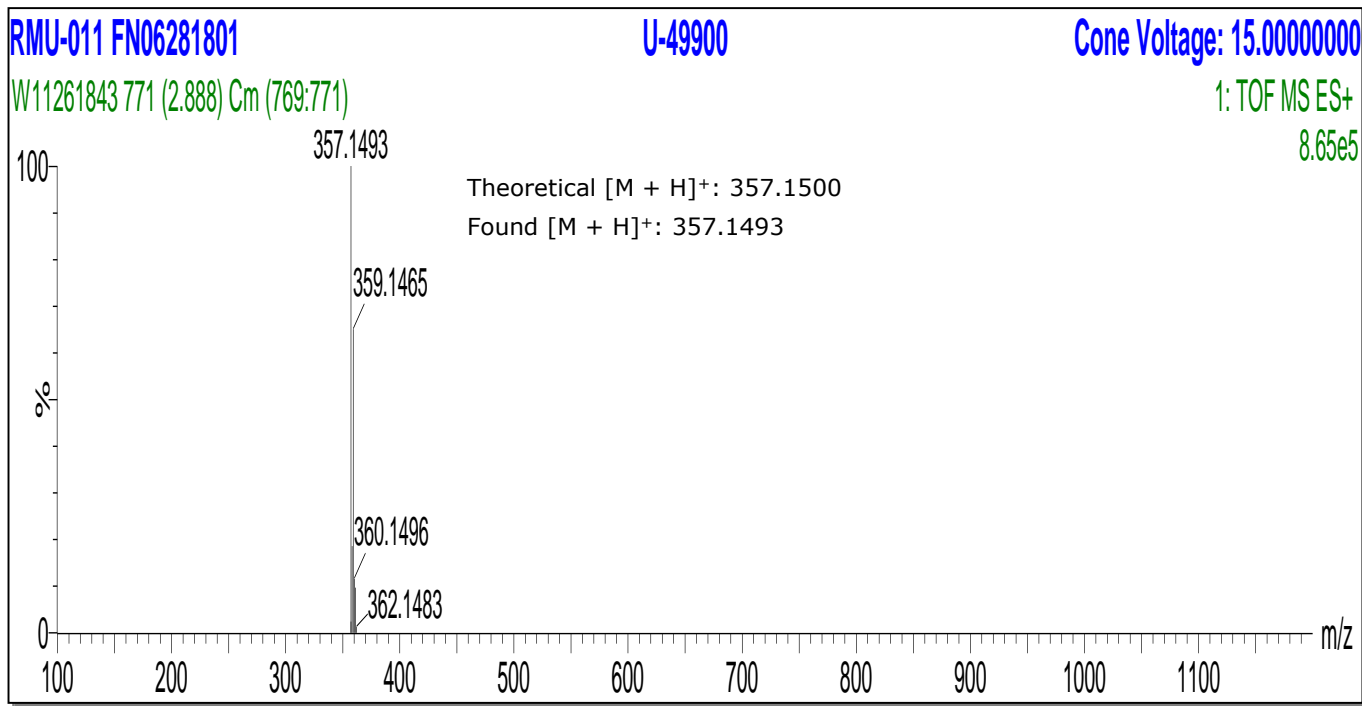
Column: Ascentis Express C18, 2.7 μ m,
3.0 x 50 mm

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
Instrument: Waters XEVO G2 QTOF
Acquired: November 26, 2018



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	0.72% decrease in purity was noted after one week.

Transport/Shipping: Ship cold.

Long Term Stability: Long term stability has been assessed for Freezer storage (-10 °C to -25 °C) conditions. Stability of a minimum of 21 months has been established through real-time stability studies.

Commutability

This standard is a solution of a pure substance in an organic solvent and is a Primary Standard. This Primary Standard is suitable for use in the preparation of calibrators and/or controls in any biological matrix. This standard is not in a biological matrix and therefore commutability to methods or standards in biological matrices does not apply.

COA Revision History

Revision No.	Date	Reason for Revision
00	February 05, 2019	Initial version.
01	February 08, 2019	Revised Short Term Stability section to reflect 4 week data.
02	March 15, 2020	Updated Retest Date from March 2019 to December 2020.
		Added Long Term Stability
03	September 02, 2020	Removed blank page
04	November 13, 2020	Updated Retest Date from December 2020 to October 2021.

Certified Reference Material - Certificate of Analysis

U-48800-¹³C₃, ¹⁵N₂, Primary Measurement Standard

(±)-trans-2,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methyl-benzeneacetamide-¹³C₃, ¹⁵N₂

Product No.: U-014-1ML
Lot No.: FN12261803
Description of CRM: U-48800-¹³C₃, ¹⁵N₂ HCl in Methanol (Solution)
 Nominal concentration is adjusted for HCl content.
Retest Date: August 2022 See Stability Section
Storage: Store unopened in freezer (-10 °C to -25 °C).
Shipping: Ambient. See Stability Section
Chemical formula: C₁₄¹³C₃H₂₄¹⁵N₂OCl₂•HCl
CAS No.: NA

Cerilliant Quality

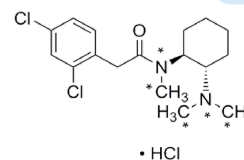
ISO 17034

ISO/IEC 17025

ISO 13485

ISO 14001

ISO 9001



Analyte	Certified Concentration ± associated uncertainty U, u=k*u (k=2)
U-48800- ¹³ C ₃ , ¹⁵ N ₂	1.000 ± 0.006 mg/mL

Metrological traceability: Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. See "Details on metrological traceability" on page 3.

Measurement method: The certified value is calculated from high precision weighing of thoroughly characterized starting material. See "Details about certification process" on page 3.

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.

Minimum sample size: 1 µL for quantitative applications

Instructions for handling and correct use: Concentration is corrected for chromatographic purity, residual solvents and residual inorganics. No adjustment required before 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. Nominal concentration is adjusted for HCl content. No adjustment required before use.

For MS Applications, we advise laboratories not to mix lots during a single sequence.

Health and safety information: Danger. Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.

Accreditation: Cerilliant Corp. is accredited by the US accreditation authority ANAB as registered reference material producer AR-1353 in accordance with ISO 17034 and registered testing laboratory AT-1352 according to ISO/IEC 17025.




Darron Ellsworth, Quality Assurance Manager

August 18, 2021

Issue Date

Packaging:

2 mL amber USP Type 1 glass ampoule containing not less than 1 mL of certified solution. Ampoules are overfilled to ensure a minimum of 1 mL volume can be transferred when using a 1mL Class A volumetric pipette.

Details on starting materials:

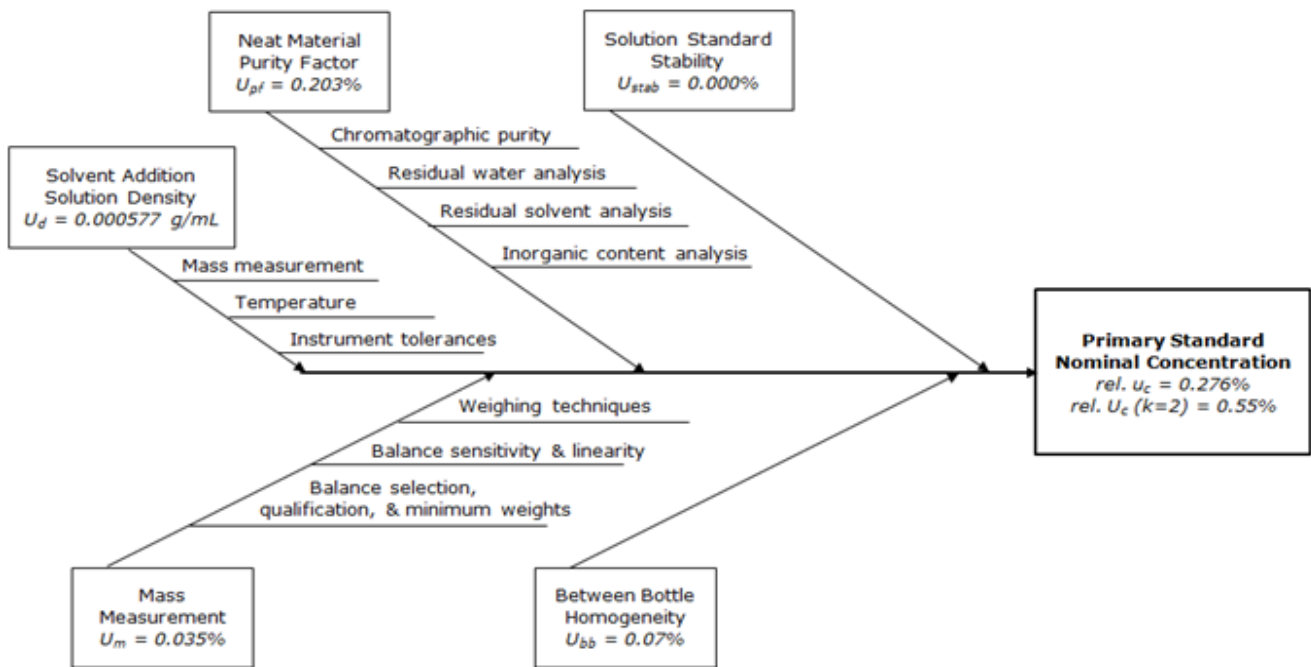
Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques and is assigned a Mass Balance Purity Factor. Spectral data is provided on subsequent pages of this CoA.

Certificate of Origin:

Cerilliant Corporation certifies no material of animal origin (BSE/TSE) was used in the preparation of this product. This material was manufactured in the USA.

Associated uncertainty:

The uncertainty has been calculated by statistical analysis of all aspects of our production system and incorporated uncertainty of the mass balance purity factor, material density, balance, weighing technique, and homogeneity. Uncertainty components of the gravimetrically prepared Primary Standard concentration are shown in the figure below. Uncertainty is expressed as an expanded uncertainty in accordance with ISO 17034 at the approximate 95% confidence interval using a coverage factor of k=2. Uncertainty contribution from neat material homogeneity was established to be negligible through establishment of process controls and verification of the control process. Stability uncertainty was determined to be negligible by regression analysis.



Details on metrological traceability:

- ♦ 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 0.10% relative error.
- ♦ Fill volume to predetermined specifications is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- ♦ The density and material Mass Balance Purity Factor of each raw material is traceable to the SI and higher order reference materials through mass measurement and instrument qualification and calibrations.

Details about certification process:

This standard has been prepared and certified under the ISO 17034, 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.

- ♦ Nominal concentration is calculated based on: the actual mass; Mass balance purity factor of the analyte(s); measured mass of the solution; and the density of the pure diluent at 20°C.
- ♦ 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.
- ♦ Additional certification information available upon request.

Solution Standard Verification

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

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

Standard Solution Assay Parameters		Calibration Curve	
Analysis Method:	HPLC/UV	Calibration Curve:	Linear Regression
Column:	Ascentis Express C18, 2.7 µm, 3.0 x 100 mm	Number of Points:	4
Mobile Phase:	Acetonitrile:0.1% Phosphoric acid in Water (40:60)	Linearity (r) :	1.000
Flow Rate:	1.5 mL/min		
Wavelength:	210 nm		
		Verified Concentration (mg/mL)	%RSD - Homogeneity
Standard Solution	Lot Number	Actual Results	Actual Results
New Lot	FN12261803	0.992	0.7
<ul style="list-style-type: none"> ♦ 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 Name:	U-48800- ¹³ C ₃ , ¹⁵ N ₂ HCl	Molecular Weight (base):	348.26
Material Lot:	FN10111802	Molecular Weight (salt):	384.72
Chemical Formula:	C ₁₄ ¹³ C ₃ H ₂₄ ¹⁵ N ₂ OCl ₂ •HCl	Salt Adjustment:	1.105
CAS Number:	NA		

Material Characterization Summary

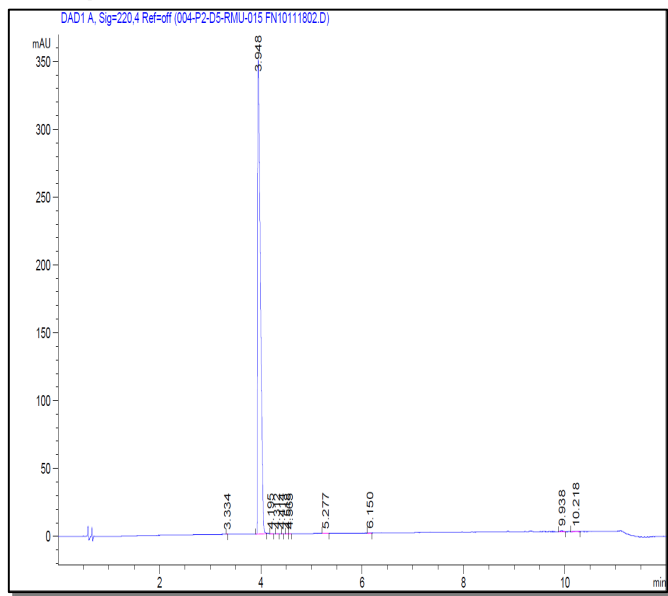
Analytical Test	Method	Results
Primary Chromatographic Purity by HPLC/UV Analysis	SP10-0102	99.7%
Secondary Chromatographic Purity by LC/MS Analysis	SP10-0107	> 99.9%
Identity by LC/MS Analysis	SP10-0107	Consistent with Structure
Isotopic Purity and Distribution by LC/MS SIM Analysis	SP10-0107	0.00% M ₀ vs M ₅
		0.00% M ₀ 0.08% M ₃
		0.03% M ₁ 1.91% M ₄
		0.01% M ₂ 97.96% M ₅
Identity by ¹ H-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure
Residual Solvent Analysis by GC/FID Headspace	AM1087 ¹	0.16%
Residual Water Analysis by Karl Fischer Coulometry	AM1346 ¹	Below Quantitation Limit
Inorganic Content by Microash Analysis	SP10-0135	< 0.2%
Mass Balance Purity Factor		99.53%

¹ Validated analytical method

- ♦ 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 purity method was selected to optimize resolution of impurities while minimizing degradation of the analyte. Secondary purity methods with orthogonal detector capabilities from the primary purity method are used as controls to confirm an accurate purity value.
- ♦ 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.

Spectral and Physical Data

HPLC/UV



Column: Ascentis Express C18, 2.7 μ m, 3.0 x 100 mm

Mobile Phase: A: Acetonitrile
B: 0.1% Phosphoric acid in Water

Gradient:

Time (min)	% A	% B
0.0	10	90
8.0	80	20
10.0	80	20
10.1	10	90

Flow Rate: 0.7 mL/min

Wavelength: 220 nm

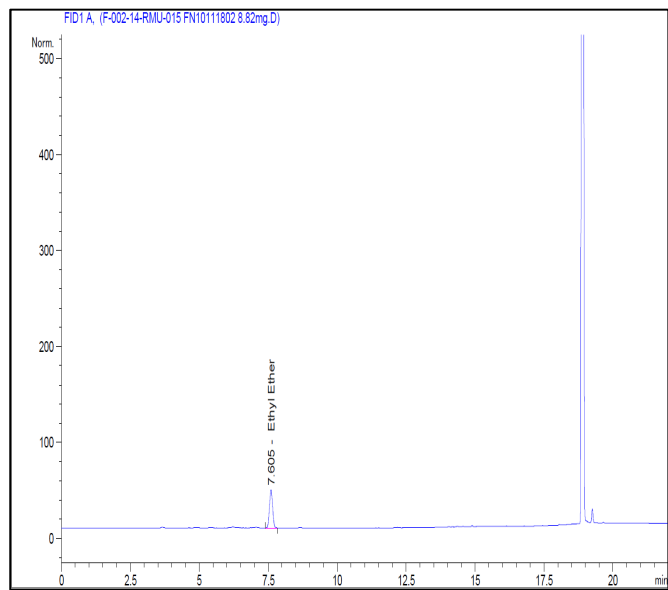
Sample Name: FN10111802

Acquired: February 11, 2019

Peak #	Ret Time	Area %
1	3.33	0.01
2	3.95	99.65
3	4.20	0.03
4	4.31	0.02
5	4.41	0.01
6	4.52	0.01
7	4.57	0.01
8	5.28	0.05
9	6.15	0.02
10	9.94	0.18
11	10.22	0.02

Spectral and Physical Data (cont.)

Residual Solvent Analysis by GC/FID Headspace



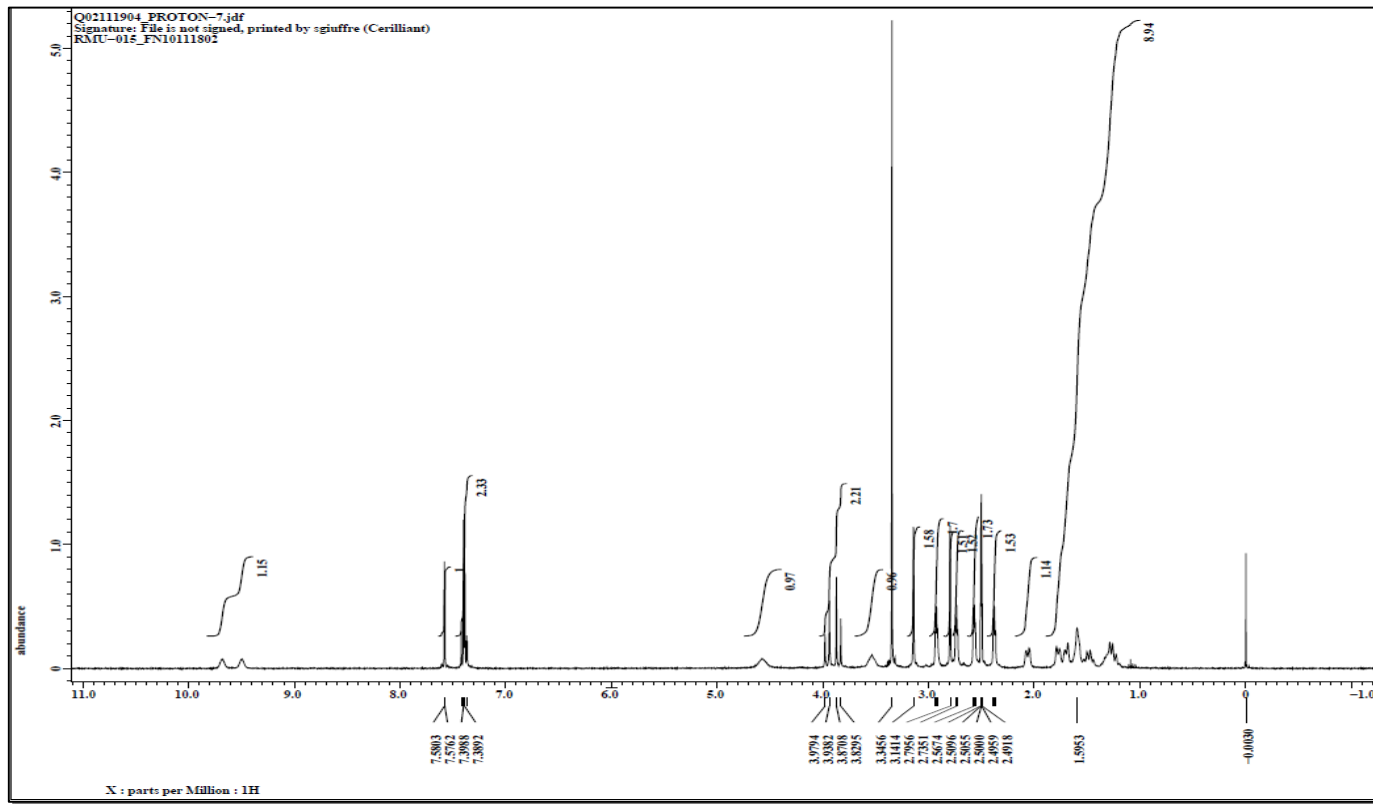
Column: DB-ALC1 30 m x 0.53 mm, 3 μm film thickness
Temp Program: 40°C (12 min) to 220°C at 40°C/min (5.5 min)
Carrier Gas: Helium
Flow Rate: 2.0 mL/min
Detector Heater Temp: 250°C
Injector: Headspace Sampler
HS Oven Temp: 60°C
Vial Equilibration: 10 minutes

Sample Name: FN10111802
Acquired: February 14, 2019

Peak	Compound	Area	Weight %
1	Ethyl ether	313.63	0.16
2	NMP	NA	NA
Total			0.16

¹H NMR

Instrument: JEOL ECS 400
Solvent: DMSO-D₆



Spectral and Physical Data (cont.)

LC/MS

Column: Ascentis Express C18, 2.7 μ m, 3.0 x 50 mm

Mobile Phase: A: 0.1% Formic acid in Water
B: Acetonitrile

Gradient:

Time (min)	% A	% B
0.0	80	20
0.5	80	20
4.0	20	80
5.8	20	80
6.0	80	20
8.0	80	20

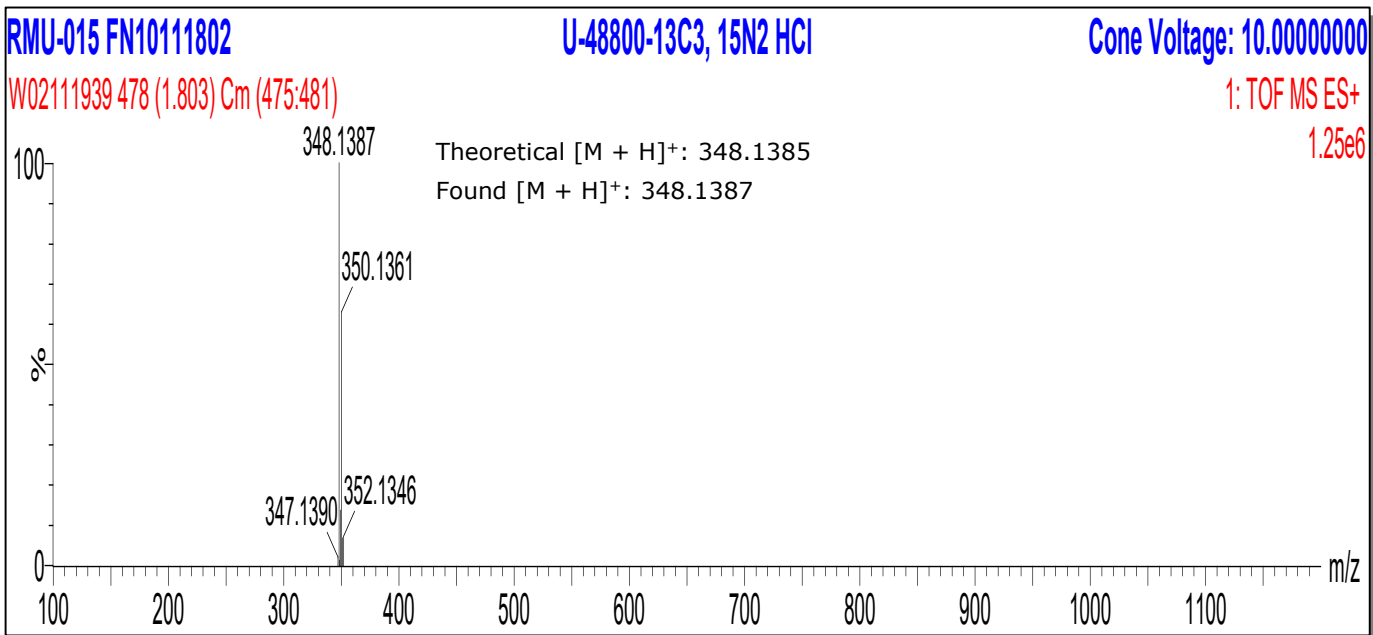
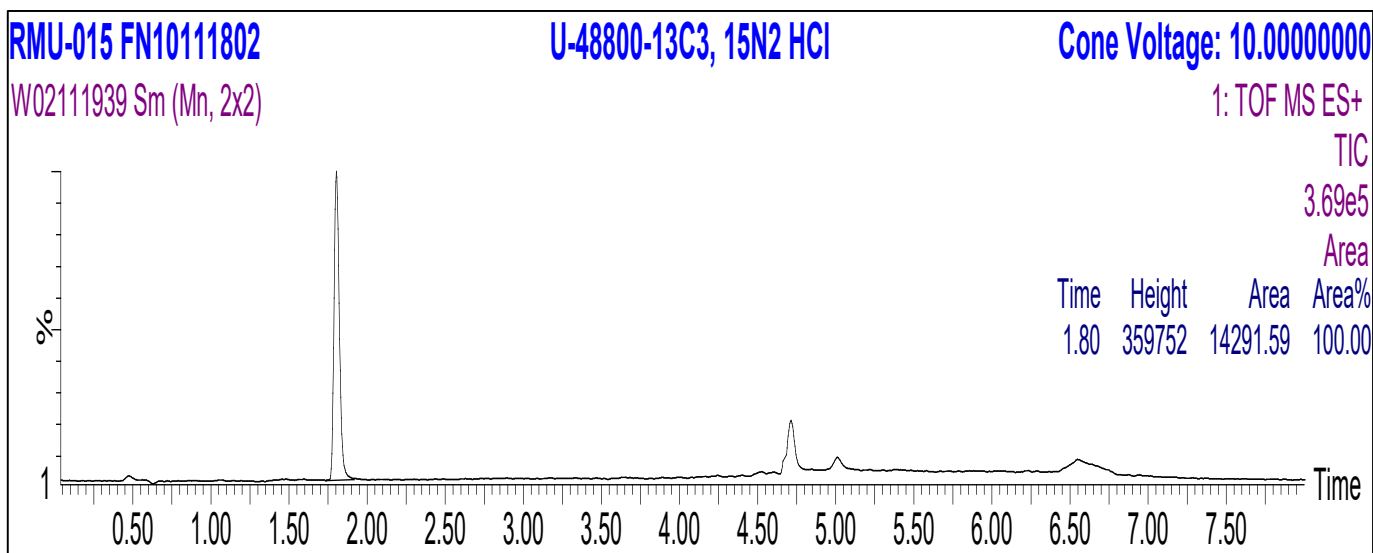
Flow Rate: 0.4 mL/min

Scan Range: 100-1200 amu

Ionization: Electrospray, Positive Ion

Instrument: Waters XEVO G2 QTOF

Acquired: February 11, 2019



Spectral and Physical Data (cont.)

Isotopic Purity by LC/MS SIM

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	80	20
0.5	80	20
4.0	20	80
5.8	20	80
6.0	80	20
8.0	80	20

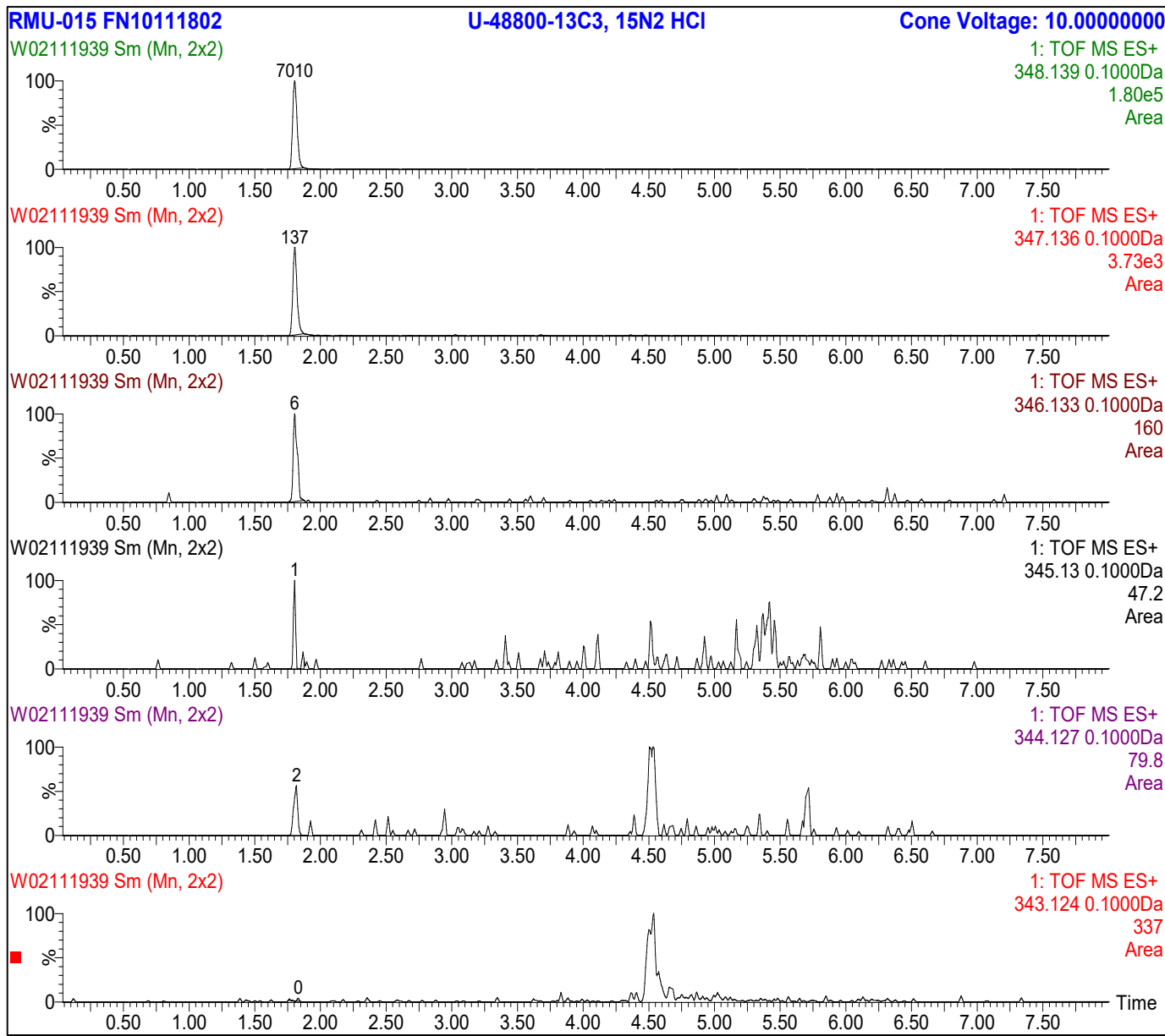
Flow Rate: 0.4 mL/min

Scan Range: 343-348 amu

Ionization: Electrospray, Positive Ion

Instrument: Waters XEVO G2 QTOF

Acquired: February 11, 2019



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 a related product (U-011-1ML, U-48800) 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.

Long Term Stability: Long term stability has been assessed for Freezer storage (-10 °C to -25 °C) conditions. Stability of a minimum of 30 months has been established through real-time stability studies.

Communtability

This standard is a solution of a pure substance in an organic solvent and is a Primary Standard. This Primary Standard is suitable for use in the preparation of calibrators and/or controls in any biological matrix. This standard is not in a biological matrix and therefore commutability to methods or standards in biological matrices does not apply.

COA Revision History

Revision No.	Date	Reason for Revision
00	February 28, 2019	Initial version.
01	April 01, 2020	Revised Retest Date from April 2020 to February 2021. Added Long Term Stability section.
02	January 04, 2021	Revised Retest Date from February 2021 to November 2021.
03	August 18, 2021	Revised Retest Date from November 2021 to August 2022.

Certified Reference Material - Certificate of Analysis

U-49900-¹³C₅, Primary Measurement Standard

(±)-*trans*-3,4-Dichloro-N-[2-(diethylamino)cyclohexyl]-N-methylbenzamide-¹³C₅

Product No.: U-015-1ML
Lot No.: FN01141903
Description of CRM: U-49900-¹³C₅ in Methanol (Solution)
Retest Date: October 2021 See Section "Stability Assessment".
Storage: Store unopened in freezer (-10 °C to -25 °C).
Shipping: Ship cold. See Section "Stability Assessment".
Chemical formula: ¹³C₅C₁₃H₂₆Cl₂N₂O
CAS No.: NA

Cerilliant Quality

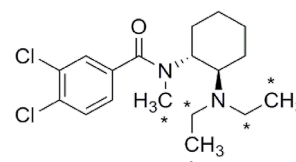
ISO 17034

ISO/IEC 17025

ISO 13485

ISO 14001

ISO 9001



Analyte	Certified Concentration ± associated uncertainty U, u=k*u (k=2)
U-49900- ¹³ C ₅	1.000 ± 0.006 mg/mL

Metrological traceability: Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. See "Details on metrological traceability" on page 2.

Measurement method: The certified value is calculated from high precision weighing of thoroughly characterized starting material. See "Details about certification process" on page 2.

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.

Minimum sample size: 1 µL for quantitative applications

Instructions for handling and correct use: Concentration is corrected for chromatographic purity, residual water, residual solvents and residual inorganics. No adjustment required before 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. For MS Applications, we advise laboratories not to mix lots during a single sequence.

Health and safety information: Danger. Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.

Accreditation: Cerilliant Corp. is accredited by the US accreditation authority ANAB as registered reference material producer AR-1353 in accordance with ISO 17034 and registered testing laboratory AT-1352 according to ISO/IEC 17025.



Darron Ellsworth, Quality Assurance Manager

October 27, 2020

Issue Date

Packaging:

2 mL amber USP Type 1 glass ampoule containing not less than 1 mL of certified solution. Ampoules are overfilled to ensure a minimum of 1 mL volume can be transferred when using a 1mL Class A volumetric pipette.

Details on starting materials:

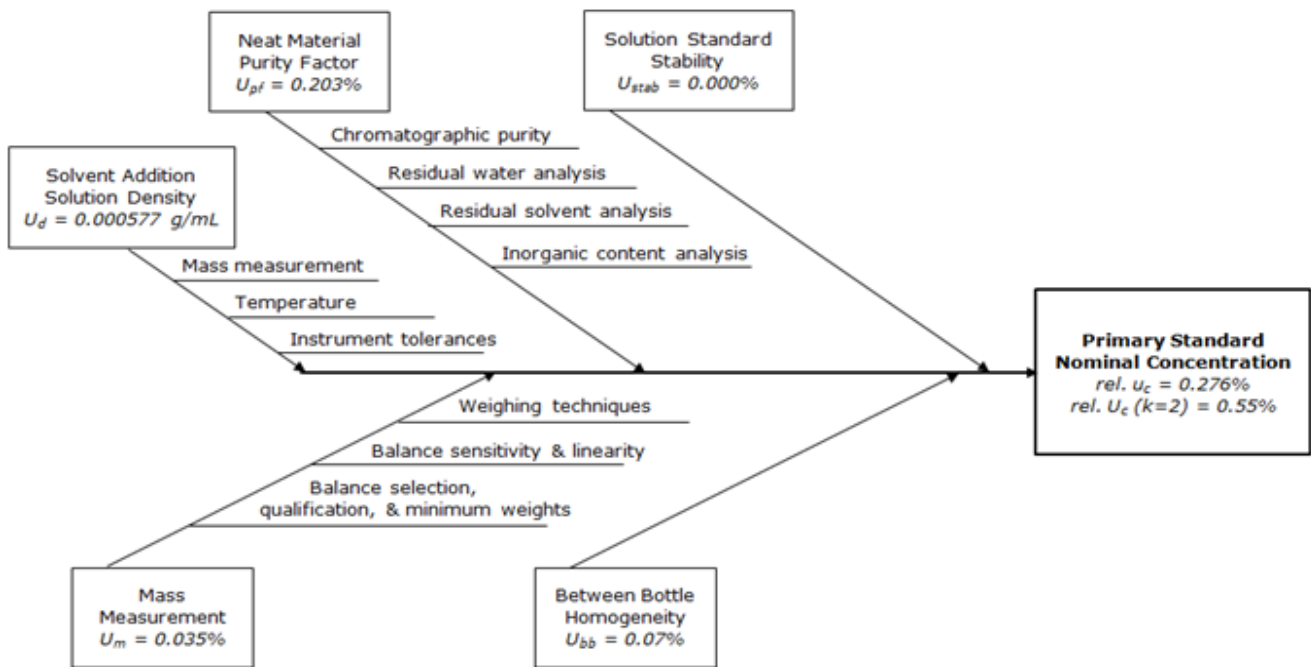
Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques and is assigned a Mass Balance Purity Factor. Spectral data is provided on subsequent pages of this CoA.

Certificate of Origin:

Cerilliant Corporation certifies no material of animal origin (BSE/TSE) was used in the preparation of this product. This material was manufactured in the USA.

Associated uncertainty:

The uncertainty has been calculated by statistical analysis of all aspects of our production system and incorporated uncertainty of the mass balance purity factor, material density, balance, weighing technique, and homogeneity. Uncertainty components of the gravimetrically prepared Primary Standard concentration are shown in the figure below. Uncertainty is expressed as an expanded uncertainty in accordance with ISO 17034 at the approximate 95% confidence interval using a coverage factor of k=2. Uncertainty contribution from neat material homogeneity was established to be negligible through establishment of process controls and verification of the control process. Stability uncertainty was determined to be negligible by regression analysis.



Details on metrological traceability:

- ♦ 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 0.10% relative error.
- ♦ Fill volume to predetermined specifications is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- ♦ The density and material Mass Balance Purity Factor of each raw material is traceable to the SI and higher order reference materials through mass measurement and instrument qualification and calibrations.

Details about certification process:

This standard has been prepared and certified under the ISO 17034, 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.

- ♦ Nominal concentration is calculated based on: the actual mass; Mass balance purity factor of the analyte(s); measured mass of the solution; and the density of the pure diluent at 20°C.
- ♦ 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.
- ♦ Additional certification information available upon request.

Solution Standard Verification

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

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

Standard Solution Assay Parameters		Calibration Curve	
Analysis Method:	HPLC/UV	Calibration Curve:	Linear Regression
Column:	Ascentis Express C18, 2.7 µm, 3.0 x 100 mm	Number of Points:	4
Mobile Phase:	Acetonitrile:0.1% Phosphoric acid in Water (40:60)	Linearity (r) :	1.000
Flow Rate:	1.5 mL/min		
Wavelength:	210 nm		
		Verified Concentration (mg/mL)	%RSD - Homogeneity
Standard Solution	Lot Number	Actual Results	Actual Results
New Lot	FN01141903	0.996	1.1
<ul style="list-style-type: none"> ♦ 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 is utilized to calculate the weighing adjustment necessary to ensure accuracy of the solution standard concentration.

Material Name:	U-49900- ¹³ C ₅	Chemical Formula:	¹³ C ₅ C ₁₃ H ₂₆ Cl ₂ N ₂ O
Material Lot:	FN10181801	CAS Number:	NA
		Molecular Weight:	362.28

Material Characterization Summary

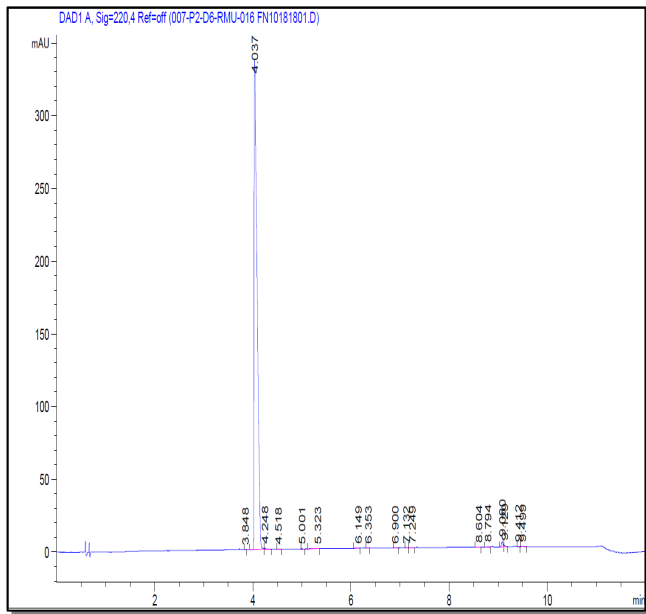
Analytical Test	Method	Results
Primary Chromatographic Purity by HPLC/UV Analysis	SP10-0102	98.9%
Secondary Chromatographic Purity by GC/FID Analysis	SP10-0101	99.4%
Identity by LC/MS Analysis	SP10-0107	Consistent with Structure
Isotopic Purity and Distribution by LC/MS SIM Analysis	SP10-0107	0.04% ¹³ C ₀ vs ¹³ C ₅
		0.04% ¹³ C ₀ 0.04% ¹³ C ₃
		0.00% ¹³ C ₁ 2.65% ¹³ C ₄
		0.02% ¹³ C ₂ 97.26% ¹³ C ₅
Identity by ¹ H-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure
Residual Solvent Analysis by GC/FID Headspace	AM1087 ¹	0.41%
Residual Water Analysis by Karl Fischer Coulometry	AM1346 ¹	Below Quantitation Limit
Inorganic Content by Microash Analysis	SP10-0135	< 0.2%
Mass Balance Purity Factor		98.51%

¹ Validated analytical method

- ♦ 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 purity method was selected to optimize resolution of impurities while minimizing degradation of the analyte. Secondary purity methods with orthogonal detector capabilities from the primary purity method are used as controls to confirm an accurate purity value.
- ♦ 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.

Spectral and Physical Data

HPLC/UV



Column: Ascentis Express C18, 2.7 μ m, 3.0 x 100 mm

Mobile Phase: A: Acetonitrile
B: 0.1% Phosphoric acid in Water

Gradient:

Time (min)	% A	% B
0.0	10	90
8.0	80	20
10.0	80	20
10.1	10	90

Flow Rate: 0.7 mL/min

Wavelength: 220 nm

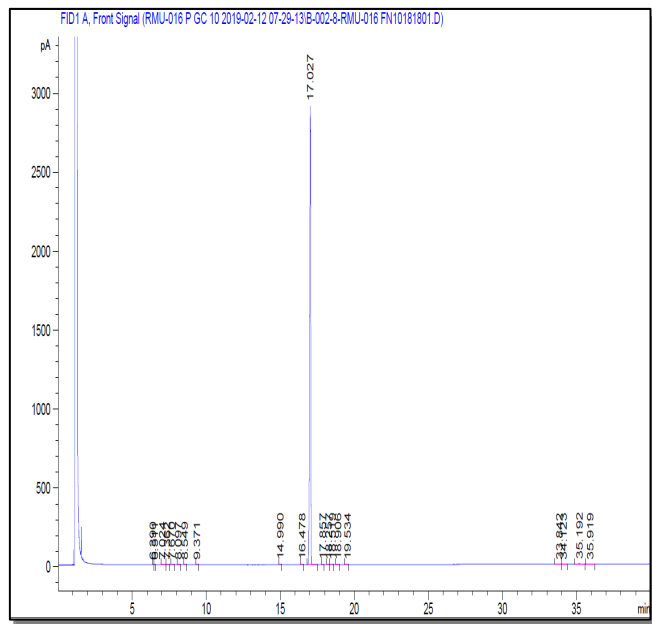
Sample Name: FN10181801

Acquired: February 11, 2019

Peak #	Ret Time	Area %
1	3.85	0.01
2	4.04	98.79
3	4.25	0.14
4	4.52	0.02
5	5.00	0.01
6	5.32	0.03
7	6.15	0.02
8	6.35	0.03
9	6.90	0.02
10	7.13	0.03
11	7.25	0.02
12	8.60	0.02
13	8.79	0.08
14	9.08	0.54
15	9.13	0.13
16	9.41	0.02
17	9.50	0.09

Spectral and Physical Data (cont.)

GC/FID

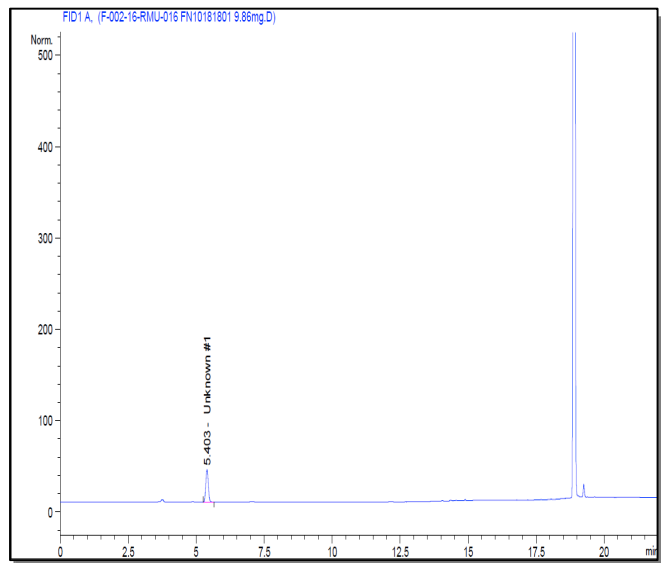


Column: DB-5ms, 30 m x 0.53 mm ID, 1.5 µm film thickness
Temp Program: 40°C to 200°C at 40°C/min
 200°C to 300°C at 5°C/min
 hold 16 min
Injector Temp: Cool-on-Column
Detector Temp: 325°C
Sample Name: FN10181801
Acquired: February 12, 2019

Peak #	Ret Time	Area %
1	6.39	0.00
2	6.51	0.00
3	7.02	0.01
4	7.36	0.02
5	7.67	0.00
6	8.10	0.00
7	8.55	0.00
8	9.37	0.00
9	14.99	0.01
10	16.48	0.01
11	17.03	99.37
12	17.86	0.02
13	18.26	0.02
14	18.52	0.05
15	18.91	0.04
16	19.53	0.02
17	33.84	0.04
18	34.12	0.05
19	35.19	0.31
20	35.92	0.03

Spectral and Physical Data (cont.)

Residual Solvent Analysis by GC/FID Headspace



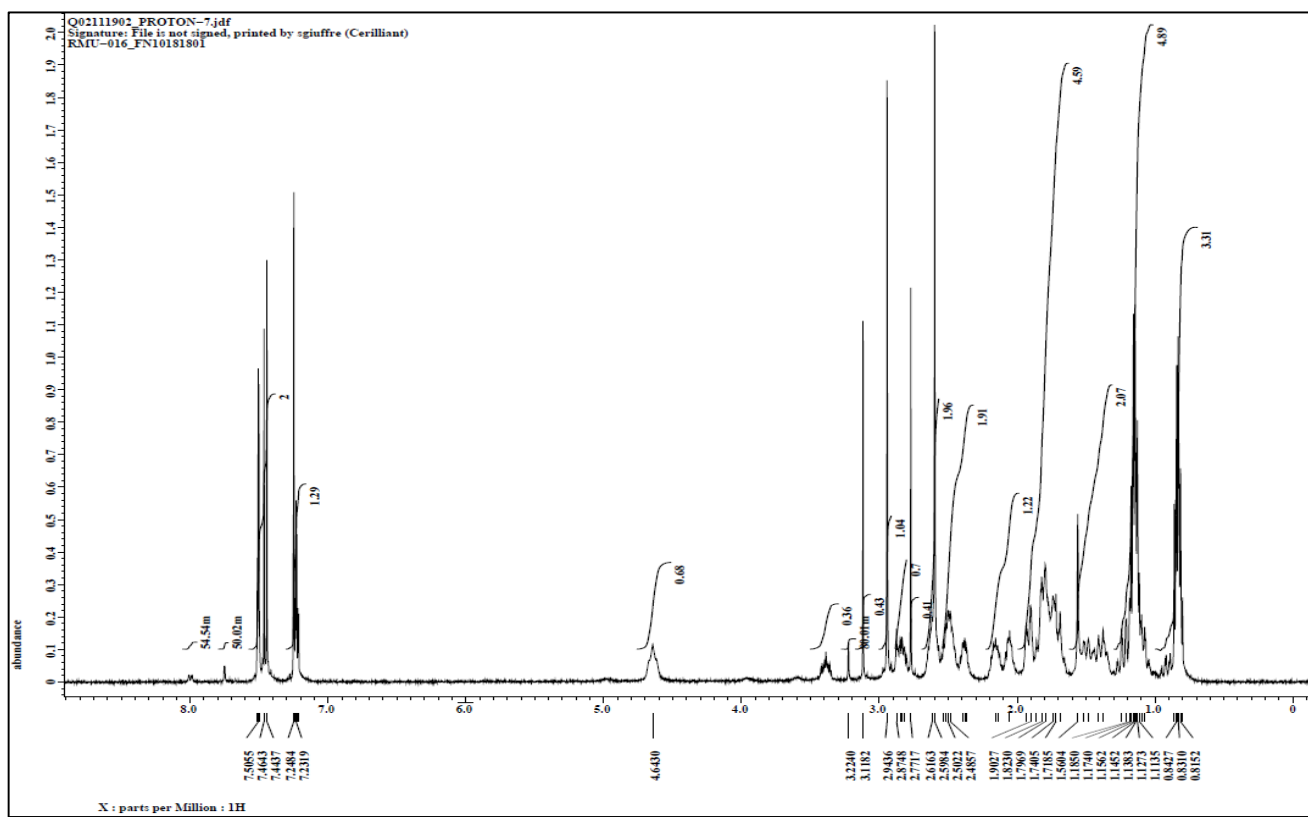
Column: DB-ALC1 30 m x 0.53 mm, 3 µm film thickness
Temp Program: 40°C hold 12 min to 220°C at 40°C/min hold 5.5 min
Carrier Gas: Helium
Flow Rate: 2.0 mL/min
Detector Heater Temp: 250°C
Injector: Headspace Sampler
HS Oven Temp: 60°C
Vial Equilibration: 10 minutes

Sample Name: FN10181801
Acquired: February 14, 2019

Peak	Compound	Area	Weight %
1	Unknown #1	228.04	0.41
2	NMP	NA	NA
Total			0.41

¹H NMR

Instrument: JEOL ECS 400
Solvent: Chloroform-D



Spectral and Physical Data (cont.)

LC/MS

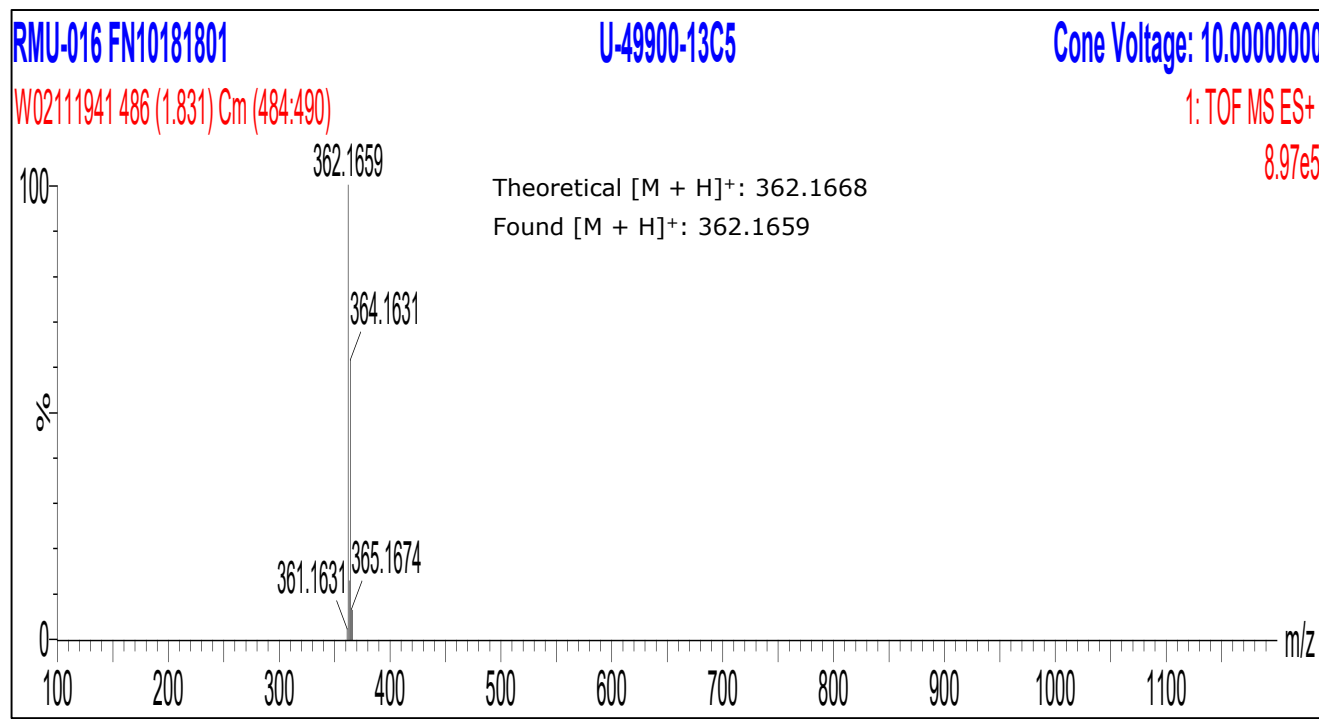
Column: Ascentis Express C18, 2.7 μ m,
3.0 x 50 mm

Mobile Phase: A: 0.1% Formic acid in Water
B: Acetonitrile

Gradient:

Time (min)	% A	% B
0.0	80	20
0.5	80	20
4.0	20	80
5.8	20	80
6.0	80	20
8.0	80	20

Flow Rate: 0.4 mL/min
Scan Range: 100-1200 amu
Ionization: Electrospray, Positive Ion
Instrument: Waters XEVO G2 QTOF
Acquired: February 11, 2019



Spectral and Physical Data (cont.)

Isotopic Purity by LC/MS SIM

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	80	20
0.5	80	20
4.0	20	80
5.8	20	80
6.0	80	20
8.0	80	20

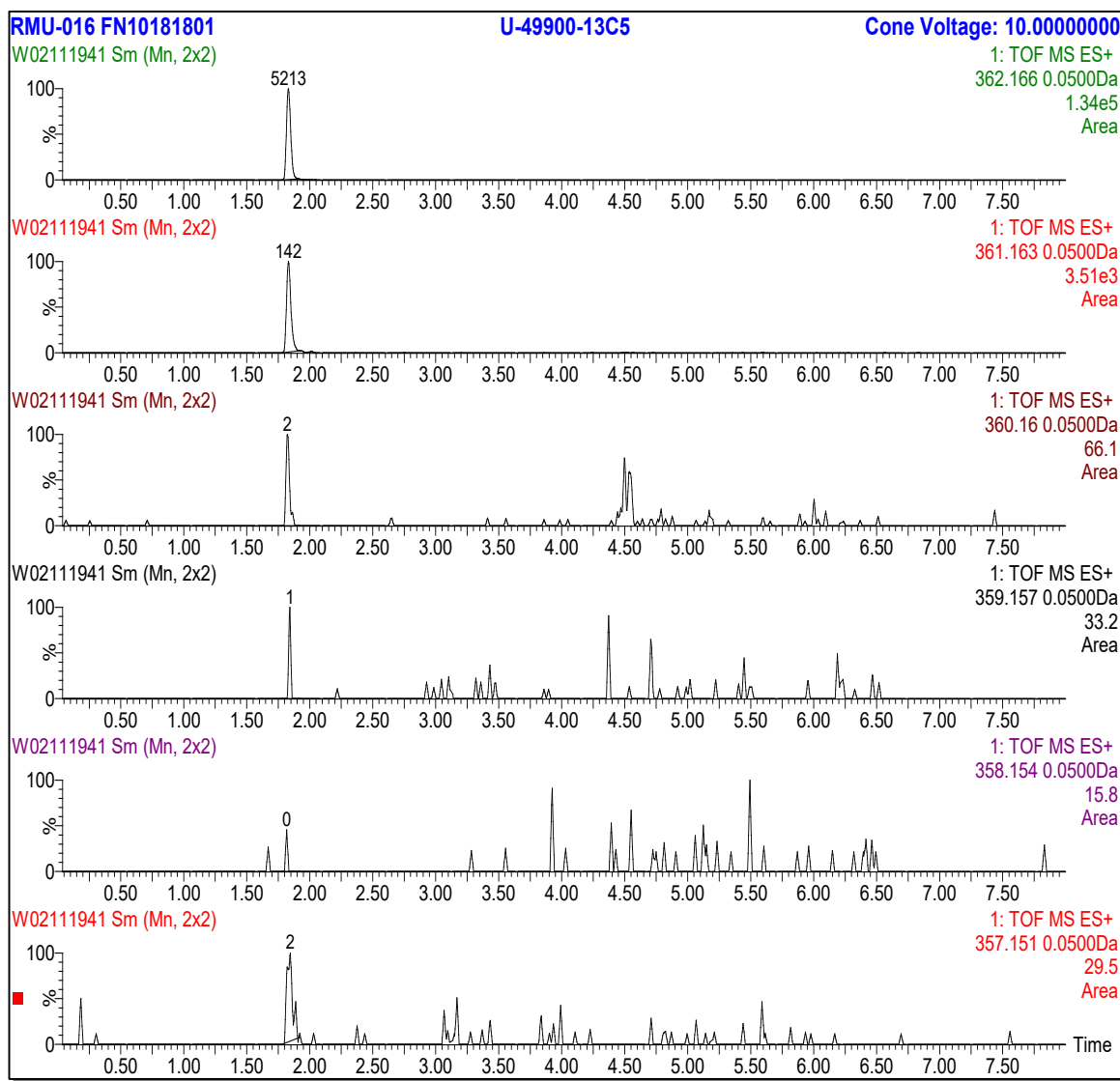
Flow Rate: 0.4 mL/min

Scan Range: 357-362 amu

Ionization: Electrospray, Positive Ion

Instrument: Waters XEVO G2 QTOF

Acquired: February 11, 2019



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 a related product (U-009-1ML, U-49900) 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	0.72% decrease in purity was noted after one week.

Transport/Shipping: Ship cold.

Long Term Stability: Long term stability has been assessed for Freezer storage (-10 °C to -25 °C) conditions. Stability of a minimum of 20 months has been established through real-time stability studies.

Commutability

This standard is a solution of a pure substance in an organic solvent and is a Primary Standard. This Primary Standard is suitable for use in the preparation of calibrators and/or controls in any biological matrix. This standard is not in a biological matrix and therefore commutability to methods or standards in biological matrices does not apply.

COA Revision History

Revision No.	Date	Reason for Revision
00	February 28, 2019	Initial version.
01	April 01, 2020	Updated Retest Date of April 2020 to January 2021.
		Added Long Term Stability section.
02	October 27, 2020	Updated Retest Date of January 2021 to October 2021.