

# Certified Reference Material - Certificate of Analysis

# $(-)-\Delta^8$ -THC, Primary Measurement Standard

(6aR,10aR)-6a,7,10,10a-tetrahydro-6,6-9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-ol

**Product No.:** T-032-1ML Lot No.: FE04282108

**Description of CRM:**  $(-)-\Delta^{8}$ -THC in Methanol (Solution)

**Expiration Date:** May 2026 See Stability Section

Storage: Store unopened in freezer (-10 °C to -25 °C).

Ambient. See Stability Section Shipping:

Chemical formula:  $C_{21}H_{30}O_2$ CAS No.: 5957-75-5

Regulatory: USDEA Exempt | Canadian TK # 61-1550  $CH_3$ OH  $CH_3$ 

Analyte	Certified Concentration $\pm$ associated uncertainty $U$ , $u = k * u  (k = 2)$
(-)-Δ <sup>8</sup> -THC	1.000 ± 0.009 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.

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 20, 2022

Issue Date



Cerilliant Corporation, 811 Paloma Drive, Suite A, Round Rock, TX, 78665, USA, Tel: 800-848-7837 / 512-238-9974; www.cerilliant.com Sigma-Aldrich Production GmbH is a subsidiary of Merck KGaA, Darmstadt, Germany. **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

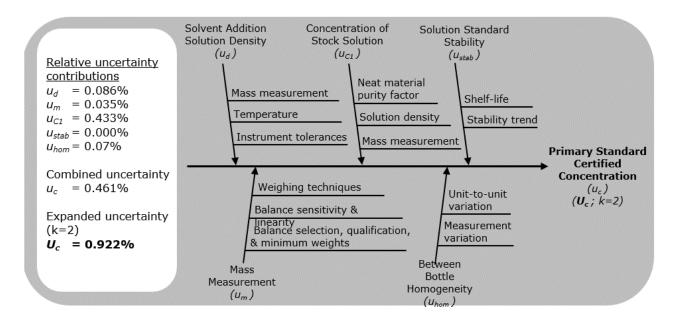
Factor. Spectral data is provided on subsequent pages of this CoA.

**Certificate of Origin:** Cerilliant Corporation certifies no material of animal origin (BSE/TSE) was used in the

preparation of this product. This material is a product of 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, and ISO 9001 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.

**Standard Solution Assay Parameters** 

**Analysis Method:** HPLC/UV

**Column:** Ascentis Express C18, 2.7 µm, 3.0 x 50 mm **Mobile Phase:** Acetonitrile:[Methanol:Water:Tetrahydrofuran

(62:33:5)] (30:70)

Flow Rate: 1.5 mL/min
Wavelength: 228 nm

**Calibration Curve** 

**Calibration Curve:** Linear Regression

Number of Points: 4 Linearity (r): 1.000

		Verified Concentration (mg/mL)	%RSD - Homogeneity
Standard Solution	Lot Number	Actual Results	Actual Results
New Lot	FE04282108	0.992	0.3
Previous Lot	FE12271903	0.990	0.2

<sup>•</sup> Concentration is verified through multiple analyses and is calculated as the average of multiple analyses compared to an independently prepared calibration solution.

<sup>•</sup> 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:(-)- $\Delta^8$ -THCChemical Formula: $C_{21}H_{30}O_2$ Material Lot:FC04181902CAS Number:5957-75-5Molecular Weight:314.46

Material Characterization Summary			
Analytical Test	Method	Results	
Primary Chromatographic Purity by HPLC/UV Analysis	AM1280	99.7% <sup>1</sup>	
Secondary Chromatographic Purity by GC/FID Analysis	AM1266	99.4%	
Chiral Purity by HPLC/UV Analysis	AM1319	97.8% ee	
Identity by GC/MS Analysis	SP10-0105	Consistent with Structure	
Identity by <sup>1</sup> H-NMR Analysis	USP <761>, SP10-0116	Consistent with Structure	
Residual Water Analysis by Karl Fischer Coulometry	AM1346 <sup>2</sup>	Not Detected	
Purity Factor Based on Assay Value		100.8%	

 $<sup>^{1}</sup>$  0.03% Cannabidiol and 0.07% (-)- $\Delta^{9}$ -THC detected by HPLC/UV analysis. No Cannabinol detected.

- A secondary chromatographic purity method is utilized as a control.
- The Mass Balance Purity Factor is based on Assay by HPLC/UV.

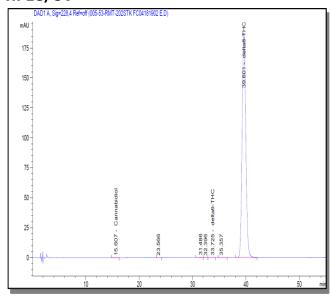
<sup>&</sup>lt;sup>2</sup> Validated analytical method

<sup>•</sup> 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.

<sup>•</sup> 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.

# Spectral and Physical Data

#### **HPLC/UV**



**Column:** Luna C18, 3 μm, 4.6 x 150 mm **Mobile Phase:** Methanol:Water:Tetrahydrofuran

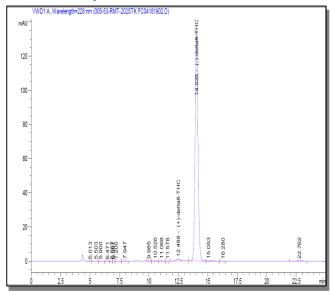
(71:24:5)

Flow Rate: 1.0 mL/min Wavelength: 228 nm

**Sample Name:** FC04181902 **Acquired:** June 04, 2019

Peak #	<b>Ret Time</b>	Area %	
1	15.61	0.03	Cannabidiol
2	23.57	0.02	
3	31.49	0.08	
4	32.40	0.05	
5	33.73	0.07	(-)-∆ <sup>9</sup> -THC
6	35.36	0.04	
7	39.60	99.71	(-)-∆ <sup>8</sup> -THC

#### **Chiral HPLC/UV**



Column: Chiralpak AD-H, 5  $\mu$ m, 4.6 x 250 mm

**Mobile Phase:** Isopropanol:Methanol:Heptane

(31:9:950)

Flow Rate: 0.7 mL/min Wavelength: 228 nm

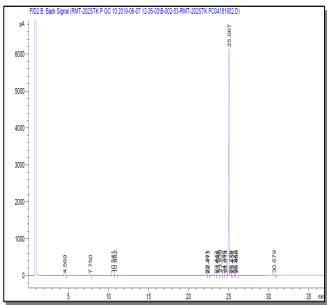
**Sample Name:** FC04181902 **Acquired:** June 13, 2019

Peak #	Ret Time	Area %	
1	5.01	0.00	<del>_</del>
2	5.50	0.02	
3	5.90	0.01	
4	6.47	0.00	
5	6.87	0.00	
6	6.97	0.00	
7	7.20	0.01	
8	7.95	0.02	
9	9.99	0.01	
10	10.53	0.05	
11	11.07	0.01	
12	11.58	0.00	
13	12.49	1.11	$(+)$ - $\Delta^8$ -THC
14	14.04	98.34	(-)-∆ <sup>8</sup> -THC
15	15.05	0.15	
16	16.28	0.01	
17	22.76	0.26	
	0/000 -	- 97 77	

ee = 97.77

# Spectral and Physical Data (cont.)

# GC/FID



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

1.0 µm film thickness

**Temp Program:** 60°C to 200°C at 10°C/min

200°C to 280°C at 5°C/min hold 7 min

Injector Temp: Cool-on-Column

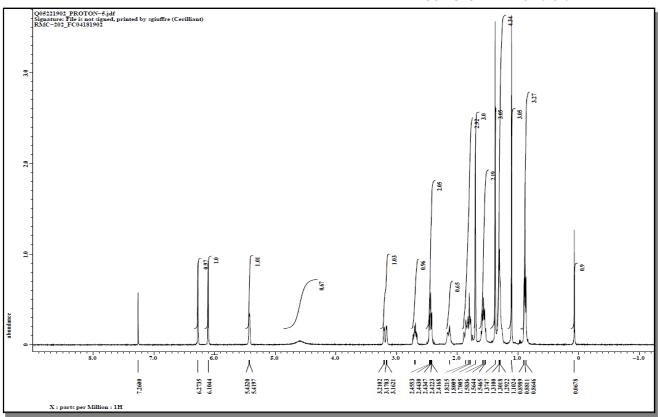
**Detector Temp:** 325°C

**Sample Name:** FC04181902 **Acquired:** June 07, 2019

Peak #	<b>Ret Time</b>	Area %
1	4.57	0.02
2	7.75	0.01
3	10.54	0.01
4	10.86	0.06
5	22.30	0.02
6	22.41	0.01
7	23.44	0.01
8	23.65	0.02
9	23.94	0.27
10	24.39	0.01
11	24.61	0.05
12	25.07	99.40
13	25.28	0.04
14	25.44	0.01
15	25.84	0.02
16	25.96	0.04
17	30.68	0.01

# Spectral and Physical Data (cont.)

1H NMRInstrument:JEOL ECS 400Solvent:Chloroform-D



#### Spectral and Physical Data (cont.)

#### GC/MS

```
Compound Name
                        : (-)-delta8-THC
Lot Number
                        : FC04181902
                        : Agilent GCMS
Instrument
Operator
                        : ECM(SGIUFFRE)
Date Reported
                       : Thu Jun 06 10:44:24 2019
                        : DB-5ms, 30m x 0.25mm ID, 0.25um film thickness
: 50°C to 200°C@40°C/min, 200°C to 300°C@10°C/min, 16min hold
Column Type
Temp. Program
Injector Temp.
                        : Cool on-column
Carrier Gas
                        : Helium
Flow Rate (mL\min) : 0.80 mL/min
Transfer Line Temp. : 280°C
Scan Range
                        : 50-500
Total Ion Chromatogram
Abundance
                                       TIC: T06061903.D\data.ms
                            11.669
 7000000
 6000000
 5000000
 4000000
 3000000
 2000000
 1000000
           6.00
                 8.00
                       10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00
Mass Spectrum
                                Scan 706 (11.659 min): T06061903.D\data.ms
Abundance
                                                      231.2
  800000
  600000
                                                                           314.3
  400000
                                                             258.1
                                            193.2
  200000
                                        174.1
                          119.1
          55.1
                                                                                     355.1
                              الإدرنجو والرحوام والويونية فإنوني والارت
       40
                      100
                          120
                               140 160 180 200 220
                                                        240
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                                                                             320
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### Stability

Short term stability studies have been performed in multiple storage conditions for a period of up to four weeks. Short term data is utilized 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 stability findings for this product is listed below.

Storage Condition	Targeted Mean Kinetic Temperature (MKT)	Time Period/Result
Freezer	-20°C	
Refrigerator	5°C	No decrease in purity was noted after
Room Temperature	20°C	four weeks.
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 60 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	January 20, 2022	Initial version.

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