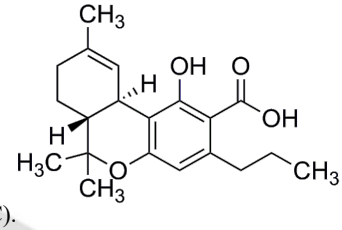
Cerilliant
Analytical Reference Standards
a SIGMA-ALDRICH company

Certified Reference Material - Certificate of Analysis

Tetrahydrocannabivarinic Acid (THCVA), Primary Measurement Standard

(6aR,10aR)-6a,7,8,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-3-propyl-6H-Dibenzo[b,d]pyran-2-carboxylic acid

Catalog Number: T-111-1ML
Lot: FE07211701
Expiration Date: April 2022
Description: Tetrahydrocannabivarinic Acid (THCVA) in Acetonitrile.
Packaging: Solution in 2 mL amber USP Type I glass ampoule containing not less than 1 mL of certified solution.
Storage: Store unopened and upright in sub-freezer (-60 °C to -80 °C).
Shipping: Ship cold. See Stability Section.
Intended Use: This Certified Reference Material is suitable for the *in vitro* identification, calibration, and quantification of the analyte(s) in analytical and R&D applications. Not suitable for human or animal consumption.
Instructions for Use: Users should quantitatively transfer desired volume using established good laboratory practices to spike into matrix or to dilute to the desired concentration. Each ampoule is intended for one-time use.
Regulatory: USDEA Exempt | Canadian TK # 61-1594 **Safety: Danger. See Safety Data Sheet**



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

- Expiration Date has been established through real time stability studies.
- Ampoules are overfilled to ensure a minimum 1 mL volume can be transferred when using a 1 mL Class A volumetric pipette.
- For quantitative applications, the minimum sample size for intended use is 1 µL.

Analyte	Certified Concentration Value
Tetrahydrocannabivarinic Acid (THCVA)	1.000 ± 0.010 mg/mL

- Uncertainty of the concentration is expressed as an expanded uncertainty in accordance with ISO 17025 and Guide 34 at the approximate 95% confidence interval using a coverage factor of k = 2 and has been calculated by statistical analysis of our production system and incorporates uncertainty of the mass balance purity factor, material density, balance, and weighing technique.
- This standard is prepared gravimetrically and mass results are reported on the conventional basis for weighing in air. Nominal concentration is calculated based on: the actual measured mass; Mass Balance Purity Factor of the analyte(s); measured mass of the solution; and the density of the pure diluent at 20 °C.
- Concentration is corrected for chromatographic purity, residual water, residual solvents and residual inorganics. No adjustment required before use.
- Additional certification information available upon request.

Metrological Traceability

- This standard has been prepared and certified under the ISO Guide 34, ISO/IEC 17025, ISO 9001 and ISO 13485 standards. This standard meets the requirements of a Certified Reference Material and a Primary Measurement Standard as defined by ISO and is traceable to the SI and higher order standards through an unbroken chain of comparisons.
- This standard has been gravimetrically prepared using balances that have been fully qualified and calibrated to ISO 17025 requirements. All calibrations utilize NIST traceable weights which are calibrated externally by a qualified ISO 17025 accredited calibration laboratory to NIST standards. Qualification of each balance includes the assignment of a minimum weighing by a qualified and ISO 17025 accredited calibration vendor taking into consideration the balance and installed environmental conditions to ensure compliance with USP tolerances of NMT 0.10% relative error.
- Fill volume is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- Concentration is verified against an independently prepared calibration solution gravimetrically prepared.
- Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques. Spectral data is provided on subsequent pages of this COA. The density and material Mass Balance Purity Factor is traceable to the SI and higher order reference standards through mass measurement and instrument qualification and calibrations.

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

ANAB ACCREDITED ISO GUIDE 34 REFERENCE MATERIAL PRODUCER

Darron Ellsworth, Quality Assurance Manager

March 29, 2023
Date

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

Analysis Method:	HPLC/UV
Column:	Ascentis Express C18, 2.7 µm, 3.0 x 50 mm
Mobile Phase:	Acetonitrile:0.1% Phosphoric acid in Water (67:33)
Flow Rate:	1.2 mL/min
Wavelength:	225 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**	**Acceptance Criteria**	**Actual Results**	**Acceptance Criteria**
New Lot	FE07211701	0.997	± 3%	0.3	≤ 3%

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

Analyte Certification - Mass Balance Purity Factor

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

Material Name:	Tetrahydrocannabivarinic Acid (THCVA)	**Chemical Formula:**	$C_{20}H_{26}O_4$
Material Lot:	FC02171702	**CAS Number:**	39986-26-0
		Molecular Weight:	330.42

Material Characterization Summary

Analytical Test	Method	Results
Primary Chromatographic Purity by HPLC/UV Analysis	SP10-0102	97.7% [1]
Secondary Chromatographic Purity by LC/MS Analysis	SP10-0107	98.6%
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 [2]	0.54%
Residual Water Analysis by Karl Fischer Coulometry	AM1346 [2]	Not Detected
Mass Balance Purity Factor		97.16%

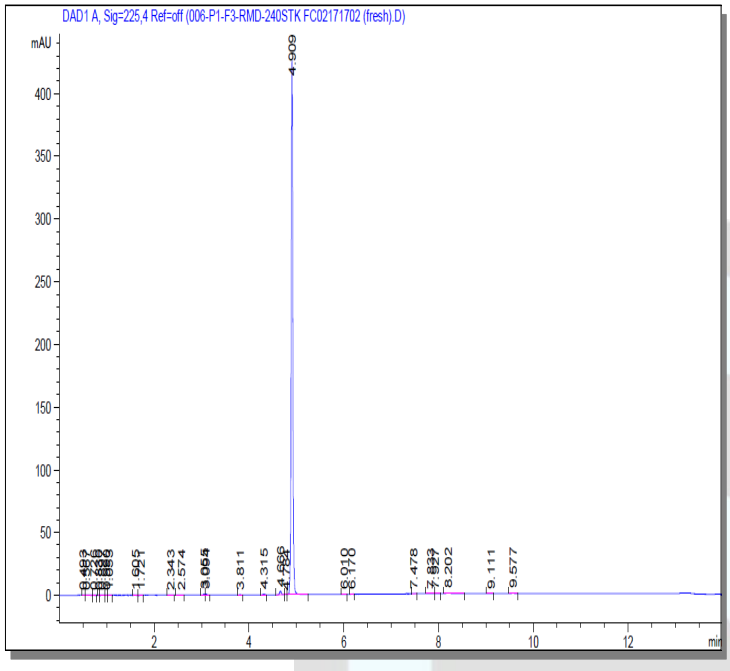
- The primary chromatographic purity is calculated as the average of two independently performed analyses utilizing two different methods. Acceptance criteria requires the purity values to be within 0.5% of each other.
- The primary chromatographic purity value is used to calculate the Mass Balance Purity Factor.
- A secondary chromatographic purity method is utilized as a control.
- Mass Balance Purity Factor = [(100 - wt% residual solvent - wt% residual water - wt% residual inorganics) x Chromatographic Purity/100].
- Mass Balance Purity Factor does not include adjustment for chiral and/or isotopic purity.

[1] 0.14% Tetrahydrocannabivarin (THCV) detected by HPLC/UV.

[2] Validated analytical method

Spectral and Physical Data

HPLC/UV



Column: Ascentis Express C18, 2.7 µm, 3.0 x 100 mm
Mobile Phase: A: Acetonitrile
B: 0.1% Phosphoric acid in Water

Gradient:

Time (min)	% A	% B
0.0	55	45
6.0	95	5
12.0	95	5
12.1	55	45

Flow Rate: 0.8 mL/min
Wavelength: 225 nm

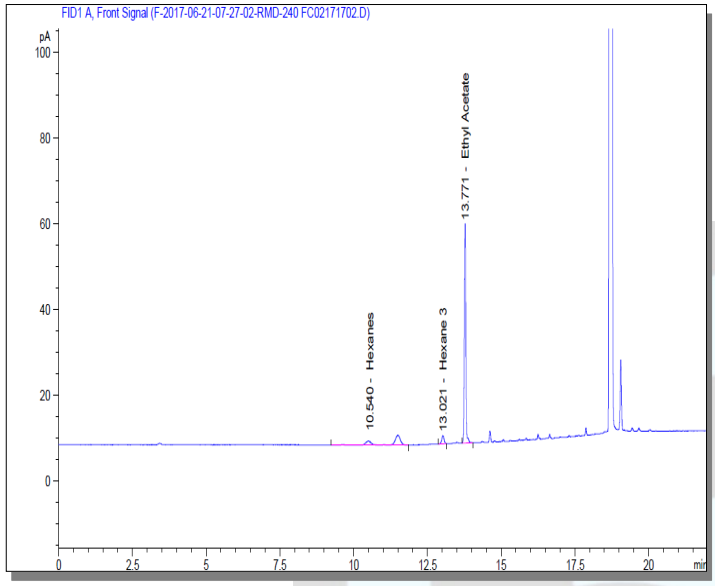
Data File Name: 2017-07-08 00-40-39\006-P1-F3.D
Instrument: LC#14
Sample Name: FC02171702
Acquired: July 08, 2017

Peak #	Ret Time	Area	Height	Area %
1	0.49	0.18	0.10	0.02
2	0.57	0.51	0.13	0.05
3	0.73	0.22	0.07	0.02
4	0.83	0.15	0.08	0.01
5	0.90	0.61	0.24	0.06
6	0.99	0.17	0.08	0.02
7	1.05	0.72	0.31	0.07
8	1.61	0.32	0.11	0.03
9	1.72	0.26	0.07	0.02
10	2.34	0.42	0.11	0.04
11	2.57	0.47	0.09	0.04
12	3.06	1.88	0.83	0.18
13	3.09	2.07	0.87	0.19
14	3.81	0.70	0.26	0.07
15	4.32	1.47	0.53	0.14
16	4.67	7.53	3.06	0.71
17	4.78	0.40	0.18	0.04
18	4.91	1032.87	425.53	97.43
19	6.01	0.31	0.08	0.03
20	6.17	0.32	0.12	0.03
21	7.48	0.27	0.09	0.03
22	7.83	1.35	0.22	0.13
23	7.93	0.43	0.11	0.04
24	8.20	4.34	0.48	0.41
25	9.11	0.53	0.15	0.05
26	9.58	1.59	0.35	0.15

Peak 15 has been identified as THCV

Spectral and Physical Data (cont.)

Residual Solvent Analysis by GC/FID Headspace

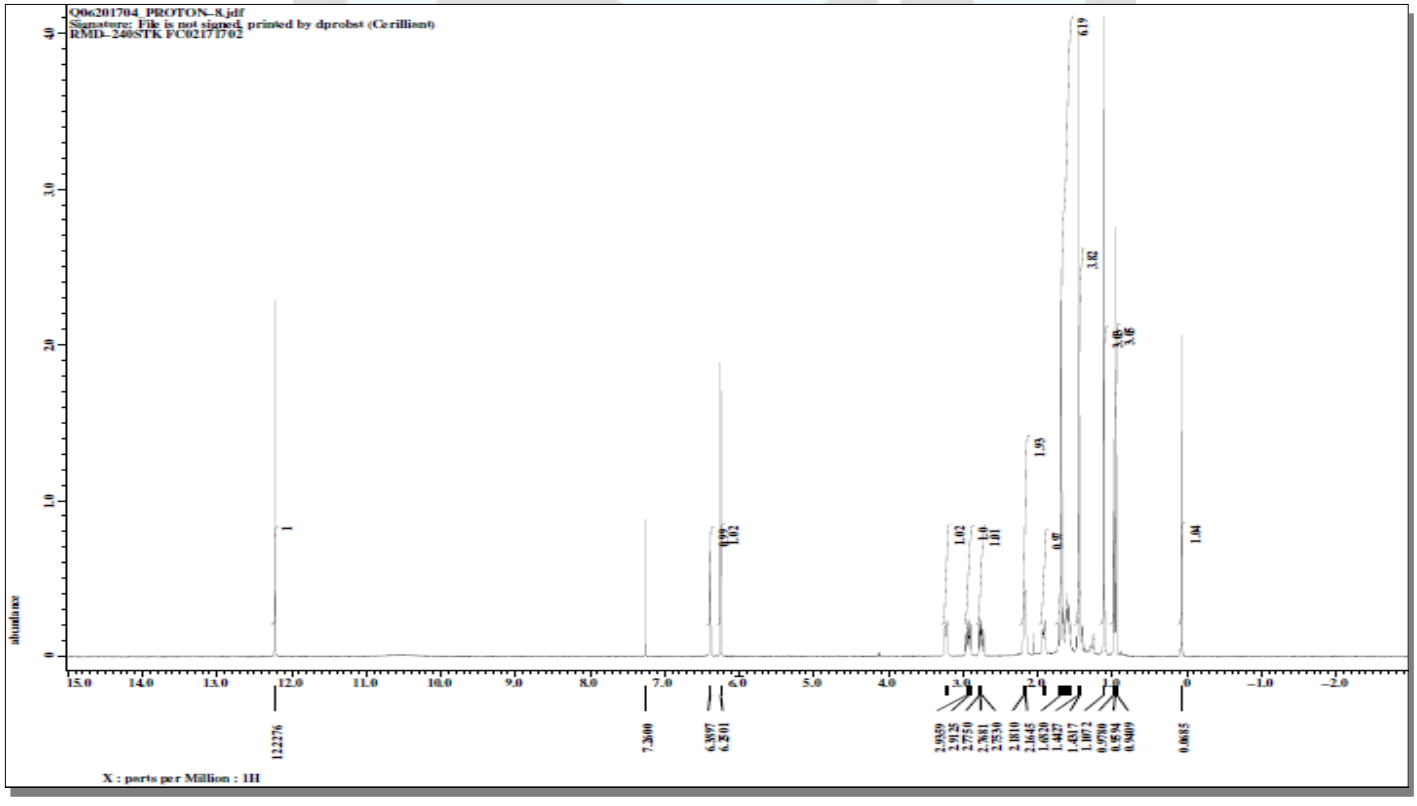


Column:	DB-ALC1 30 m x 0.53 mm, 3 µm film thickness
Temp Program:	40°C (12 min) to 220°C at 40°C/min (5.5 min)
Carrier Gas:	Helium
Flow Rate:	2.0 mL/min
Detector Heater Temp:	250°C
Injector:	Headspace Sampler
HS Oven Temp:	60°C
Vial Equilibration:	10 minutes
Data File Name:	2017-06-21 06-53-17\F-2017-06-21-07-27-02.D
Instrument:	GC#11
Sample Name:	FC02171702
Acquired:	June 21, 2017

Peak	Compound	Area	Weight %
1	Hexanes	47.03	0.01
2	Ethyl acetate	183.72	0.53
3	NMP	NA	NA
Total			**0.54**

^{1}H NMR

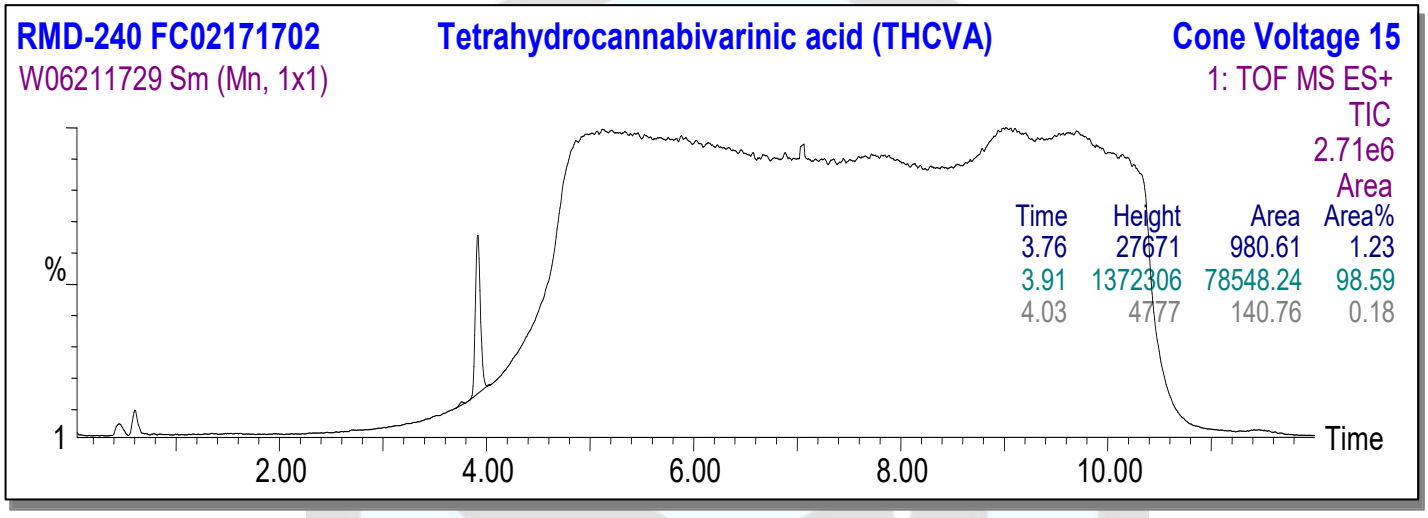
Instrument: JEOL ECS 400
Solvent: Chloroform-D

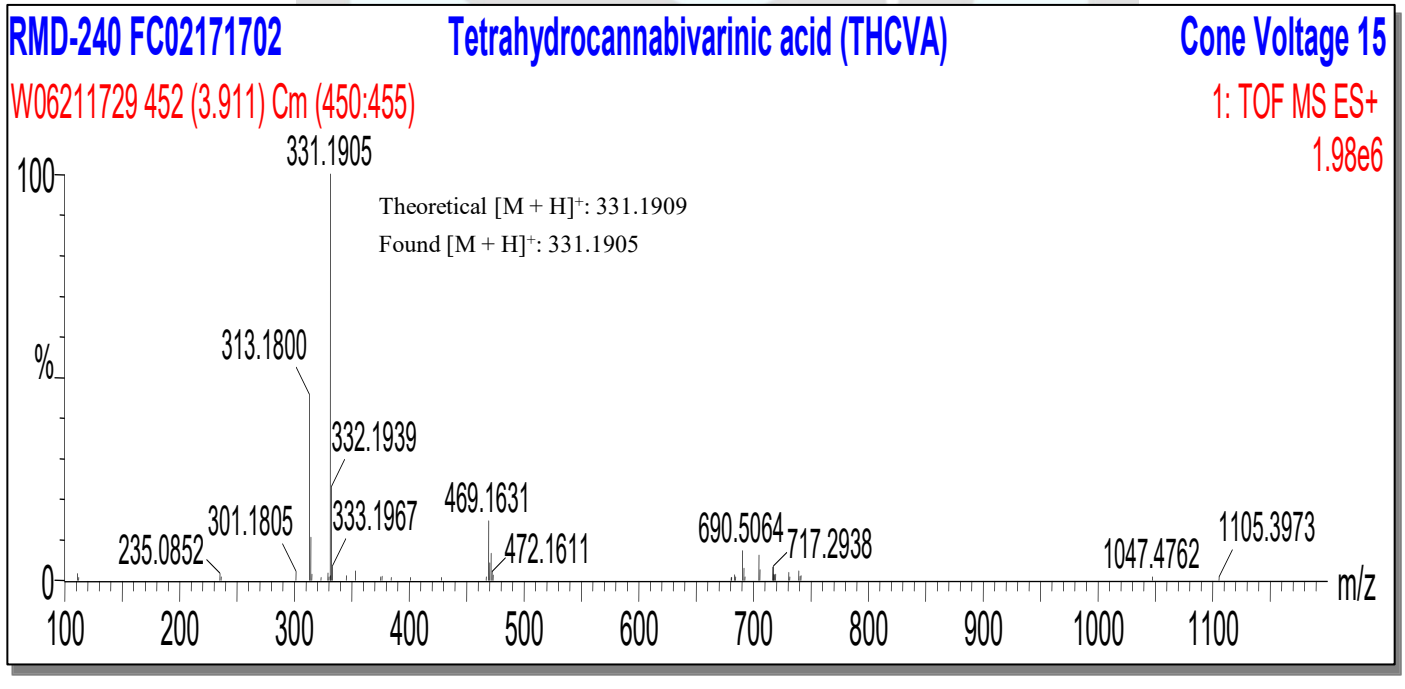


Spectral and Physical Data (cont.)

LC/MS

Column:	Ascentis Express C18, 2.7 µm, 3.0 x 50 mm			**Flow Rate:**	0.4 mL/min
Mobile Phase:	A: 0.1% Formic acid in Water			**Scan Range:**	100 - 1200 amu
	B: Acetonitile			**Ionization:**	Electrospray, Positive Ion
Gradient:	Time (min)	%A	%B	**Data File Name:**	W06211729
	0.0	50	50	**Instrument:**	Waters XEVO G2 QTOF
	4.0	10	90	**Sample Name:**	FC02171702
	9.5	10	90	**Acquired:**	June 21, 2017
	11.0	50	50		
	12.0	50	50		





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
Sub-Freezer	-70°C	No decrease in purity was noted after four weeks.
Freezer	-15°C	
Refrigerator	4°C	
Room Temperature	21°C	2.06% decrease was noted after two weeks.
40°C	40°C	7.79% decrease in purity was noted after one week.

Transport/Shipping: *Ship cold.*

Short Term Storage: *Stability data supports short term storage for no more than 12 months at Freezer conditions.*

Long Term Stability: *Long term stability has been assessed for Sub-Freezer storage (-60 °C to -80 °C) conditions. Stability of a minimum of 60 months has been established through real-time stability studies.*

COA Revision History

Revision No.	Date	Reason for Revision
00	August 25, 2017	Initial version
01	October 03, 2017	Updated Short Term Stability with four-week study data.
02	July 10, 2018	Updated Long Term Stability.
03	July 27, 2018	Revised Retest Date from October 2018 to July 2019.
		Updated Long Term Stability and Short Term Storage.
04	June 13, 2019	Revised Retest Date from July 2019 to April 2020.
05	August 01, 2019	Revised Retest Date from April 2020 to July 2020.
06	June 29, 2020	Revised Retest Date from July 2020 to April 2021.
07	July 27, 2020	Revised Retest Date from April 2021 to July 2021.
08	April 27, 2021	Revised Retest Date from July 2021 to April 2022.
09	March 29, 2023	Revised Retest Date of April 2022 to Expiration Date of April 2022.