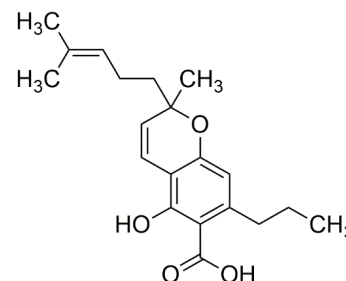


# Certified Reference Material - Certificate of Analysis

## Cannabichromevarinic acid (CBCVA), Primary Measurement Standard

*5-Hydroxy-2-methyl-2-(4-methyl-3-penten-1-yl)-7-propyl-2H-1-benzopyran-6-carboxylic acid*

**Product No.:** C-256-1ML  
**Lot No.:** FN09202202  
**Description of CRM:** Cannabichromevarinic acid (CBCVA) in 1% DIPEA and 0.05% Ascorbic acid in Acetonitrile (Solution)  
**Retest Date:** January 2025 See Stability Section  
**Storage:** Store unopened in freezer (-10 °C to -25 °C).  
**Shipping:** Ambient. See Stability Section  
**Chemical formula:** C<sub>20</sub>H<sub>26</sub>O<sub>4</sub>  
**CAS No.:** 1628112-69-5  
**Regulatory:** Canadian TK # 007-063



### Analyte

**Certified Concentration ± associated uncertainty  $U$ ,  $u = k * u$  ( $k = 2$ )**

**Cannabichromevarinic acid (CBCVA)**

**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.

**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.



  
 James Schmitt, Quality Assurance Manager

**May 21, 2024**

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:**

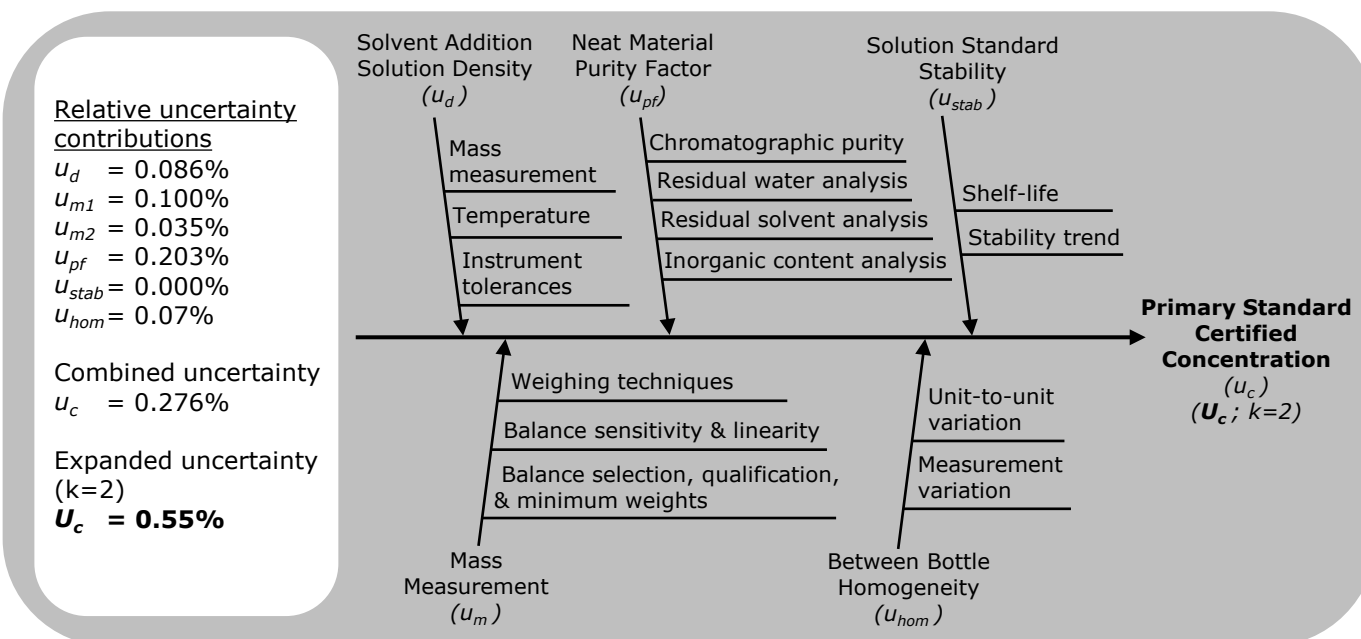
Cerilliant Corporation certifies no material of animal origin (BSE/TSE) was used in the preparation of this product.

**Country of Origin:**

United States of America

**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.



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**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.

Standard Solution Assay Parameters		Calibration Curve	
<b>Analysis Method:</b>	HPLC/UV	<b>Calibration Curve:</b>	Linear Regression
<b>Column:</b>	Ascentis Express C18, 2.7 µm, 3.0 x 50 mm	<b>Number of Points:</b>	4
<b>Mobile Phase:</b>	Acetonitrile:0.1% Phosphoric acid in Water (80:20)	<b>Linearity (r) :</b>	1.000
<b>Flow Rate:</b>	1.2 mL/min		
<b>Wavelength:</b>	254 nm		
		Verified Concentration (mg/mL)	%RSD - Homogeneity
Standard Solution	Lot Number	Actual Results	Actual Results
New Lot	FN09202202	1.014	1.7
<ul style="list-style-type: none"><li>♦ Concentration is verified through multiple analyses and is calculated as the average of multiple analyses compared to an independently prepared calibration solution.</li><li>♦ 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.</li></ul>			

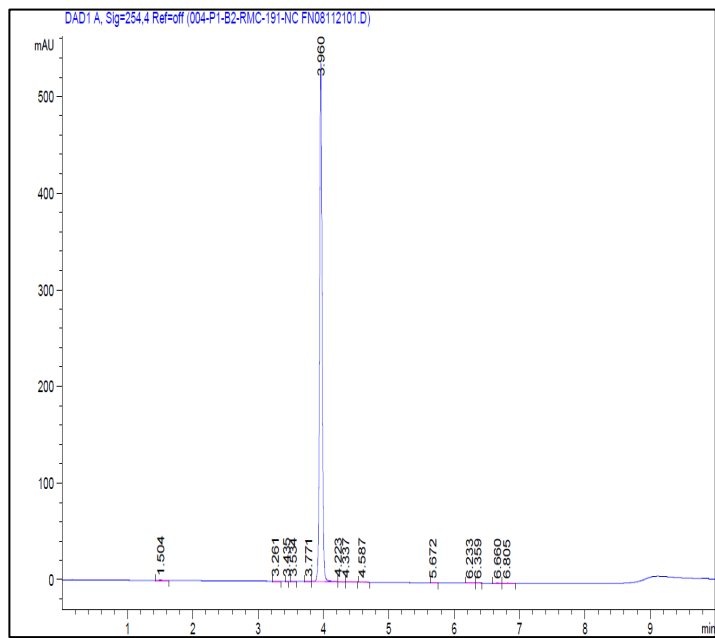
### Analyte Certification - Mass Balance Purity Factor

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.

<b>Material Name:</b>	Cannabichromevarinic acid (CBCVA)	<b>Chemical Formula:</b>	C <sub>20</sub> H <sub>26</sub> O <sub>4</sub>
<b>Material Lot:</b>	FN08112101	<b>CAS Number:</b>	1628112-69-5
		<b>Molecular Weight:</b>	330.42
<b>Material Characterization Summary</b>			
<b>Analytical Test</b>	<b>Method</b>	<b>Results</b>	
Chromatographic Purity by HPLC/UV Analysis	20384348	99.6% <sup>1</sup>	
Total THC (Δ <sup>9</sup> -THC and THCA-A) on a Dry Weight Basis	20384348	< 0.3%	
Identity by LC/MS Analysis	20384217	Consistent with Structure	
Identity by <sup>1</sup> H-NMR Analysis	20384224	Consistent with Structure	
Residual Solvent Analysis by GC/FID Headspace	20397799 <sup>2</sup>	0.04%	
Residual Water Analysis by Karl Fischer Coulometry	20384212 <sup>2</sup>	Not Detected	
Inorganic Content by Microash Analysis	20384350	Below Quantitation Limit	
Mass Balance Purity Factor		99.54%	
<div><sup>1</sup> 0.02% Cannabichromevarin (CBCV) detected by HPLC/UV analysis.</div> <div><sup>2</sup> Validated analytical method</div> <div><ul style="list-style-type: none"><li>♦ The 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.</li><li>♦ The chromatographic purity value is used to calculate the Mass Balance Purity Factor.</li><li>♦ Mass Balance Purity Factor = [(100 - wt% residual solvent - wt% residual water - wt% residual inorganics) x Chromatographic Purity/100].</li><li>♦ Mass Balance Purity Factor does not include adjustment for chiral and/or isotopic purity.</li></ul></div>			

## Spectral and Physical Data

### HPLC/UV



**Column:** Ascentis Express C18, 2.7  $\mu$ m,  
3.0 x 50 mm  
**Mobile Phase:** A: Acetonitrile  
B: 0.1% Phosphoric acid in Water  
**Gradient:**

Time (min)	% A	% B
0.0	50	50
6.0	85	15
8.0	85	15
8.1	50	50

**Flow Rate:** 1.2 mL/min  
**Wavelength:** 254 nm

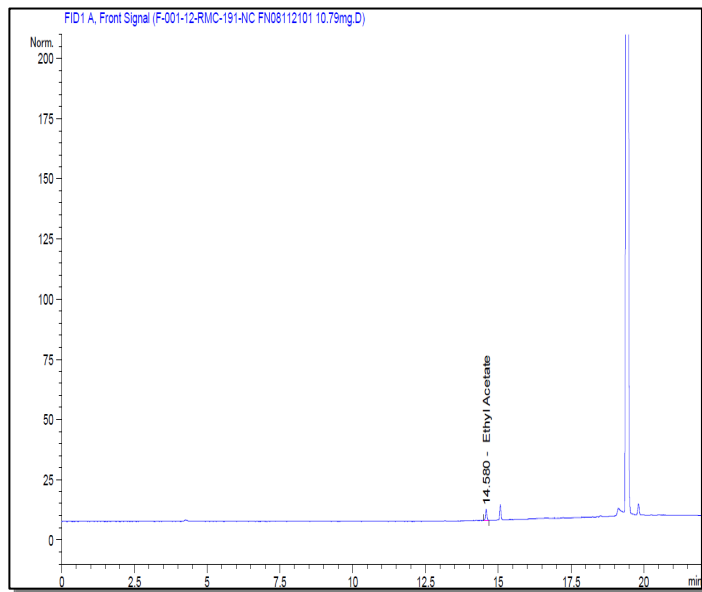
**Sample Name:** FN08112101  
**Acquired:** November 14, 2022

Peak #	Ret Time	Area %
1	1.50	0.08
2	3.26	0.02
3	3.44	0.00
4	3.53	0.02
5	3.77	0.04
6	3.96	99.57
7	4.22	0.07
8	4.34	0.04
9	4.59	0.03
10	5.67	0.01
11	6.23	0.06
12	6.36	0.02
13	6.66	0.02
14	6.81	0.02

Peak 4 is identified as CBCV

## Spectral and Physical Data (cont.)

### Residual Solvent Analysis by GC/FID Headspace



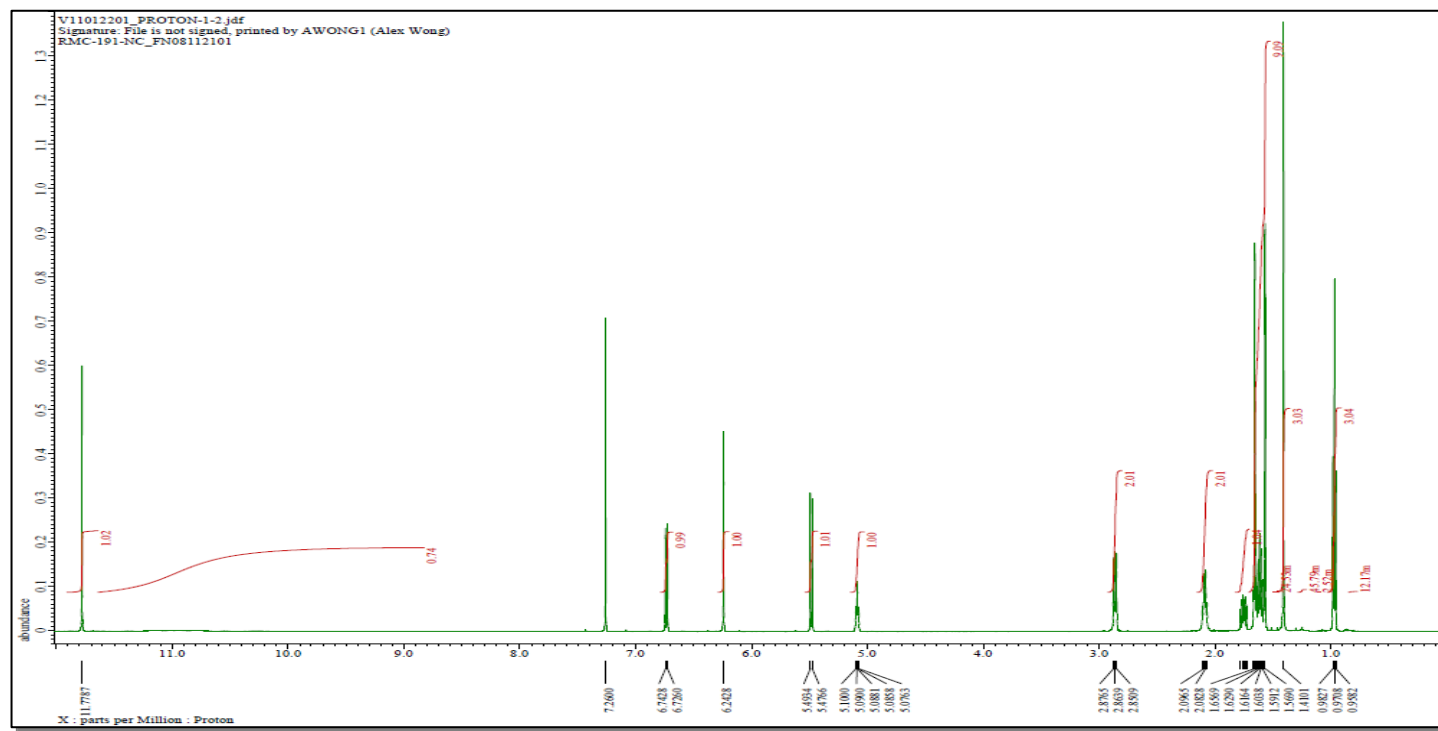
**Column:** DB-ALC1 30 m x 0.53 mm, 3 µm film thickness  
**Temp Program:** 40°C hold 12 min to 220°C at 40°C/min hold 5.5 min  
**Carrier Gas:** Helium  
**Flow Rate:** 2.0 mL/min  
**Detector Heater Temp:** 250°C  
**Injector:** Headspace Sampler  
**HS Oven Temp:** 60°C  
**Vial Equilibration:** 10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

**Sample Name:** FN08112101  
**Acquired:** November 01, 2022

Peak	Compound	Area	Weight %
1	Ethyl acetate	14.57	0.04
2	NMP	NA	NA
<b>Total</b>			<b>0.04</b>

## <sup>1</sup>H NMR

**Instrument:** JEOL ECS 400  
**Solvent:** Chloroform-D



## Spectral and Physical Data (cont.)

### LC/MS

**Column:** Ascentis Express C18, 2.7  $\mu$ m,  
3.0 x 50 mm

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

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

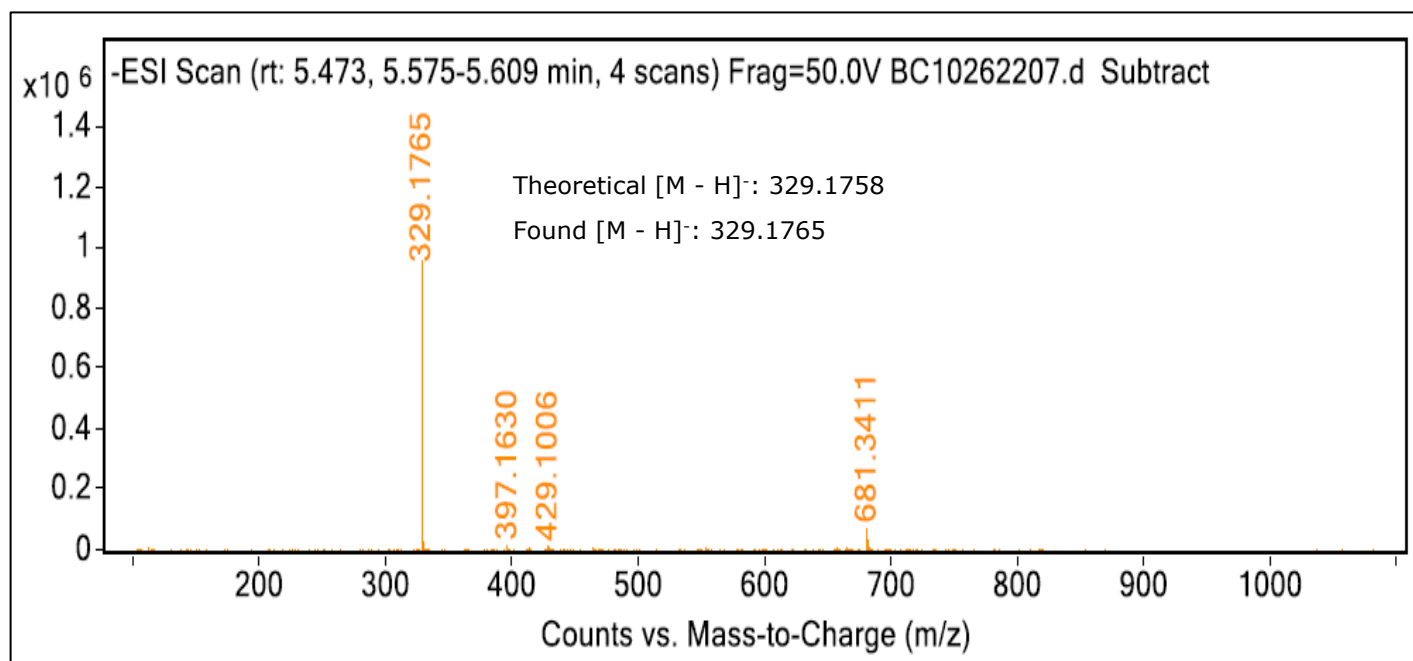
**Flow Rate:** 0.4 mL/min

**Scan Range:** 100-1200 amu

**Ionization:** Electrospray, Negative Ion

**Instrument:** Agilent 6545XT QTOF

**Acquired:** October 27, 2022





## 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	No decrease in purity was noted after four weeks.
Refrigerator	5°C	
Room Temperature	30°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 12 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	March 24, 2023	Initial version.
01	May 12, 2023	Added Regulatory section.
02	January 31, 2024	Revised Retest Date from January 2024 to January 2025.
		Added Long Term Stability data.
03	May 21, 2024	Revised Quality Assurance Manager signature.

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