



Certificate of Analysis

Certified Reference Material

HEMP-1

Certified Reference Material of dried, ground hemp

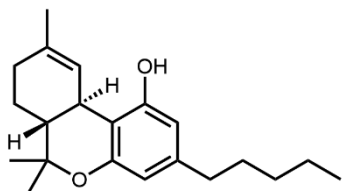
HEMP-1 is a certified reference material (CRM) designed for use in method development, validation, and quality control for the analysis of cannabinoids in cannabis and hemp. While cannabinoid solution reference standards are appropriate for instrument calibration, matrix CRMs such as HEMP-1 can help validate entire methods, including sampling, extraction, clean-up, and instrumental analysis. Certified values for the mass fraction of cannabinoids in HEMP-1 have been established, as listed in Table 1.

The certified values represent the mass fraction of cannabinoids in HEMP-1 based on results from data generated at the National Research Council of Canada (NRC). Values were assigned using two liquid chromatography – tandem mass spectrometry (LC–MS/MS) methods [1], modified with narrower calibration ranges to improve precision and accuracy. The expanded uncertainty (U) for all values is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the JCGM Guide [2] and k is the coverage factor of two ($k = 2$, 95 % confidence interval, CI). It is intended that the U for certified values accounts for every aspect that reasonably contributes to their uncertainties.

Table 1: Certified values and expanded uncertainties for HEMP-1 ($k = 2$, 95 % CI)

Substance	Symbol	Molecular formula	Mass fraction mg/g
Δ^9 -tetrahydrocannabinol (a)	Δ^9 -THC	$C_{21}H_{30}O_2$	0.289 ± 0.078
Δ^9 -tetrahydrocannabinolic acid (a)	Δ^9 -THCA	$C_{22}H_{30}O_4$	0.991 ± 0.084
total Δ^9 -tetrahydrocannabinol ¹ (a)	Total Δ^9 -THC	-	1.16 ± 0.14
cannabidiol (a)	CBD	$C_{21}H_{30}O_2$	5.10 ± 0.66
cannabidiolic acid (a)	CBDA	$C_{22}H_{30}O_4$	14.1 ± 0.8
total cannabidiol ¹ (a)	Total CBD	-	17.5 ± 1.2
cannabigerol (a)	CBG	$C_{21}H_{32}O_2$	0.0406 ± 0.0080
cannabigerolic acid (a)	CBGA	$C_{22}H_{32}O_4$	0.112 ± 0.010
cannabinol (a)	CBN	$C_{21}H_{26}O_2$	0.479 ± 0.068
cannabinolic acid (a)	CBNA	$C_{22}H_{26}O_4$	0.342 ± 0.034
cannabichromene (a)	CBC	$C_{21}H_{30}O_2$	0.291 ± 0.076
cannabichromenic acid (a)	CBCA	$C_{22}H_{30}O_4$	0.418 ± 0.100
tetrahydrocannabivarin (a)	THCV	$C_{19}H_{26}O_2$	0.0134 ± 0.0020
tetrahydrocannabivarinic acid (a)	THCVA	$C_{20}H_{26}O_4$	0.0731 ± 0.0062
cannabidivarin (a)	CBDV	$C_{19}H_{26}O_2$	0.177 ± 0.030
cannabidivarinic acid (a)	CBDVA	$C_{20}H_{26}O_4$	0.718 ± 0.054
cannabicyclol (a)	CBL	$C_{21}H_{30}O_2$	0.0707 ± 0.0128
cannabicyclolic acid (a)	CBLA	$C_{22}H_{30}O_4$	0.209 ± 0.020

¹Total cannabinoid values are expressed as total neutral cannabinoid equivalents.

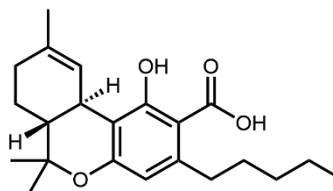
**Δ⁹-tetrahydrocannabinol (Δ⁹-THC)**

CAS registry number: 1972-08-3

InChI Key: CYQFCXCEBYINGO-IAGOWNOFSA-N

Molecular formula: C₂₁H₃₀O₂

Molar mass: 314.46 ± 0.02 g/mol

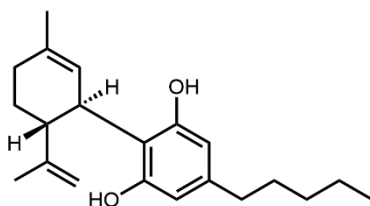
**Δ⁹-tetrahydrocannabinolic acid (Δ⁹-THCA)**

CAS registry number: 23978-85-0

InChI Key: UCONUSSAWGCZMV-HZPDHXFCSA-N

Molecular formula: C₂₂H₃₀O₄

Molar mass: 358.47 ± 0.02 g/mol

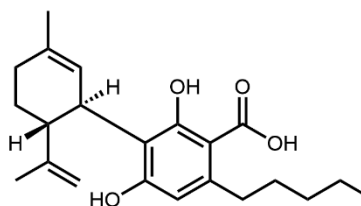
**cannabidiol (CBD)**

CAS registry number: 13956-29-1

InChI Key: QHMBSVQNZZTUGM-ZWKOTPCHSA-N

Molecular formula: C₂₁H₃₀O₂

Molar mass: 314.46 ± 0.02 g/mol

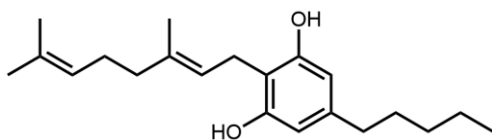
**cannabidiolic acid (CBDA)**

CAS registry number: 1244-58-2

InChI Key: WVOLTBSCXRRQFR-DLBZAZTESA-N

Molecular formula: C₂₂H₃₀O₄

Molar mass: 358.47 ± 0.02 g/mol

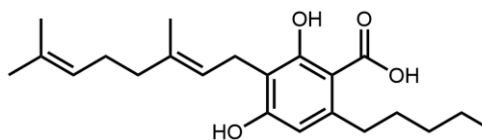
**cannabigerol (CBG)**

CAS registry number: 25654-31-3

InChI Key: QXACEHWTBCFNSA-SFQUDFHCSA-N

Molecular formula: C₂₁H₃₂O₂

Molar mass: 316.48 ± 0.02 g/mol

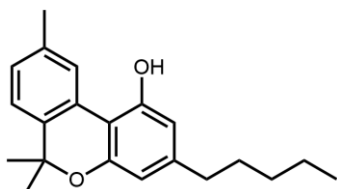
**cannabigerolic acid (CBGA)**

CAS registry number: 25555-57-1

InChI Key: SEEZIOZEUUMJME-FOWTUZBSSA-N

Molecular formula: C₂₂H₃₂O₄

Molar mass: 360.49 ± 0.02 g/mol

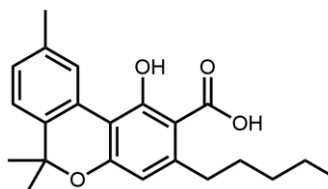
**cannabinol (CBN)**

CAS registry number: 521-35-7

InChI Key: VBGLYOIFKLUMQG-UHFFFAOYSA-N

Molecular formula: C₂₁H₂₆O₂

Molar mass: 310.43 ± 0.02 g/mol

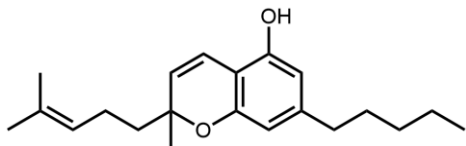
**cannabinolic acid (CBNA)**

CAS registry number: 2808-39-1

InChI Key: KXKOBIRSQLNUPS-UHFFFAOYSA-N

Molecular formula: C₂₂H₂₆O₄

Molar mass: 354.43 ± 0.02 g/mol

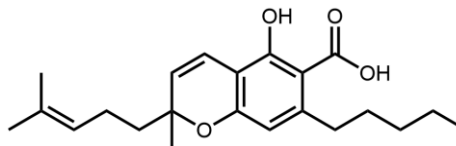
**cannabichromene (CBC)**

CAS registry number: 20675-51-8

InChI Key: UVOLYTDXHDXWJU-UHFFFAOYSA-N

Molecular formula: C₂₁H₃₀O₂

Molar mass: 314.46 ± 0.02 g/mol

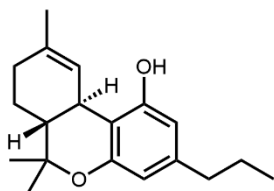
**cannabichromenic acid (CBCA)**

CAS registry number: 185505-15-1

InChI Key: HRHJHXJQMNWQTF-UHFFFAOYSA-N

Molecular formula: C₂₂H₃₀O₄

Molar mass: 358.47 ± 0.02 g/mol

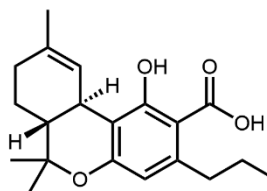
**tetrahydrocannabivarin (THCV)**

CAS registry number: 31262-37-0

InChI Key: ZROLHBHDLIHEMS-HUUCEWRRSA-N

Molecular formula: C₁₉H₂₆O₂

Molar mass: 286.41 ± 0.02 g/mol

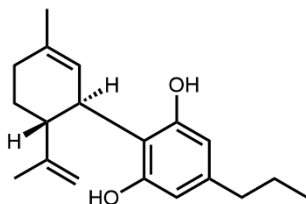
**tetrahydrocannabivarinic acid (THCVA)**

CAS registry number: 39986-26-0

InChI Key: IQSYWEWTWDEVNO-ZIAGYGMSSA-N

Molecular formula: C₂₀H₂₆O₄

Molar mass: 330.42 ± 0.02 g/mol

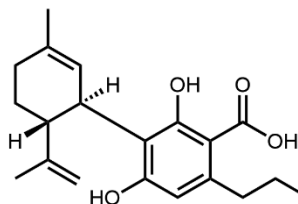
**cannabidivarin (CBDV)**

CAS registry number: 24274-48-4

InChI Key: REOZWEGFPHTFEI-JKSUJKDBSA-N

Molecular formula: C₁₉H₂₆O₂

Molar mass: 286.41 ± 0.02 g/mol

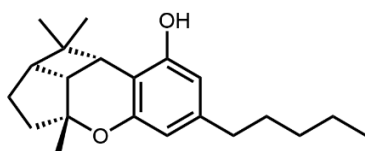
**cannabidivarinic acid (CBDVA)**

CAS registry number: 31932-13-5

InChI Key: CZXWOKHVLNYAHI-LSDHHAUSA-N

Molecular formula: C₂₀H₂₆O₄

Molar mass: 330.42 ± 0.02 g/mol

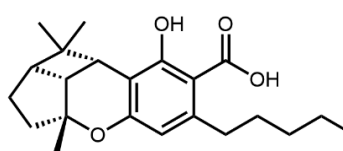
**cannabicyclol (CBL)**

CAS registry number: 21366-63-2

InChI Key: IGHTZQUIFGUJTG-UHFFFAOYSA-N

Molecular formula: C₂₁H₃₀O₂

Molar mass: 314.46 ± 0.02 g/mol

**cannabicyclolic acid (CBLA)**

CAS registry number: 40524-99-0

InChI Key: JVOHLEIRDMVLHS-UHFFFAOYSA-N

Molecular formula: C₂₂H₃₀O₄

Molar mass: 358.47 ± 0.02 g/mol

Table 2: Information values for HEMP-1

Element	Mass fraction µg/g
arsenic (b)	1.4
beryllium (b)	0.21
cadmium (b)	0.10
chromium (b)	10
cobalt (b)	1.5
lead (b)	2.6
manganese (b)	293
molybdenum (b)	0.52
nickel (b)	4.7
selenium (b)	0.18
uranium (b)	0.45

Coding

The coding refers to the instrumental method used for value assignment.

- a** Liquid chromatography – tandem mass spectrometry (LC–MS/MS)
- b** Inductively coupled plasma – mass spectrometry (ICP–MS)

Certified values

Certified values are considered to be those for which the NRC has the highest confidence in accuracy and that all known and suspected sources of bias have been taken into account and are reflected in the stated expanded uncertainties. Certified values are the best estimate of the true value and uncertainty (Table 1).

Information values

Information values are those for which insufficient data are available to provide a comprehensive estimate of uncertainty (Table 2).

Intended use

This certified reference material is primarily intended for use in method development, validation, and quality control for the analysis of Δ^9 -THC, Δ^9 -THCA, CBD, CBDA, CBG, CBGA, CBN, CBNA, CBC, CBCA, THCV, THCVA, CBDV, CBDVA, CBL, and CBLA in cannabis and hemp.

Storage

At a minimum, the material should be stored away from light in a controlled cold temperature environment such as a freezer at $-20\text{ }^{\circ}\text{C}$. For maximum stability, storage at $-80\text{ }^{\circ}\text{C}$ is recommended.

Instructions for use

Prior to opening, each bottle should be allowed to warm to room temperature and the contents should be thoroughly mixed. After use, bottles should be tightly sealed and immediately returned to the freezer.

Repeated sampling is permitted, although care must be taken not to introduce contamination. A minimum sample mass of 500 mg is recommended.

Preparation of material

HEMP-1 was prepared and homogenized by drum mixing seven containers of commercially sourced, dried, ground hemp material prior to being bottled in 15 g aliquots.

Stability

The effects of freeze-thaw (F/T) cycles, stability during transport, and long-term stability of the cannabinoids in HEMP-1 were assessed at the NRC using LC-MS/MS. Freeze-thaw stability was assessed over twenty F/T cycles from $-20\text{ }^{\circ}\text{C}$ to $+20\text{ }^{\circ}\text{C}$. The results were evaluated as a function of the number of F/T cycles using ordinary least squares fitting and indicated no significant instability trend for all cannabinoids. Therefore, the uncertainty due to F/T stability was considered negligible and set to zero.

Transportation and long-term stability studies were carried out using an isochronous approach. Bottles of HEMP-1 were stored at temperatures ranging from $-20\text{ }^{\circ}\text{C}$ to $+40\text{ }^{\circ}\text{C}$ for up to two years and compared to reference samples stored at $-80\text{ }^{\circ}\text{C}$. Degradation of each cannabinoid was modelled using a network of third-order reactions [3] and the results were evaluated using Bayesian model fitting [4]. The uncertainty components due to transportation and long-term stability were taken as the projected bias in the mass fractions of the cannabinoids between the $-80\text{ }^{\circ}\text{C}$ reference conditions and $+20\text{ }^{\circ}\text{C}$ over 2 weeks and at $-20\text{ }^{\circ}\text{C}$ over 5 years, respectively. The transportation stability uncertainty represents a conservative estimate to include possible shipping delays. These uncertainties were combined to assign an uncertainty related to stability.

Homogeneity

The material was tested for homogeneity at the NRC using LC-MS/MS. Results from subsamples (500 mg) from randomly selected bottles were evaluated using Bayesian analysis of variance (ANOVA) [4] to determine both within-unit and between-unit heterogeneity components. These uncertainties were combined to assign an associated uncertainty component.

Uncertainty

Included in the combined uncertainty estimate (u_c) are the uncertainties in the batch characterization (u_{char}), uncertainties related to within-unit and between-unit variation (u_{hom}), uncertainties related to stability ($u_{\text{stability}}$), and uncertainties related to the different methods (u_{method}). Expressed as standard uncertainties, these components are listed in Table 3.

Table 3: Uncertainty components of the certified values for HEMP-1

Substance	$U_{k=2}$ mg/g	u_c mg/g	u_{char} mg/g	u_{hom} mg/g	$u_{\text{stability}}$ mg/g	u_{method} mg/g
Δ^9 -THC	0.078	0.039	0.003	0.010	0.036	0.009
Δ^9 -THCA	0.084	0.042	0.006	0.032	0.027	0.000
Total Δ^9 -THC	0.14	0.07	0.01	0.04	0.06	0.01
CBD	0.66	0.33	0.03	0.13	0.30	0.00
CBDa	0.8	0.4	0.2	0.3	0.1	0.0
Total CBD	1.2	0.6	0.2	0.4	0.3	0.0
CBG	0.0080	0.0040	0.0005	0.0010	0.0038	0.0000

Substance	$U_{k=2}$ mg/g	U_c mg/g	U_{char} mg/g	U_{hom} mg/g	$U_{stability}$ mg/g	U_{method} mg/g
CBGA	0.010	0.005	0.001	0.003	0.004	0.002
CBN	0.068	0.034	0.003	0.009	0.032	0.000
CBNA	0.034	0.017	0.004	0.009	0.015	0.000
CBC	0.076	0.038	0.002	0.006	0.037	0.000
CBCA	0.100	0.050	0.004	0.004	0.049	0.009
THCV	0.0020	0.0010	0.0002	0.0003	0.0008	0.0004
THCVA	0.0062	0.0031	0.0006	0.0026	0.0014	0.0008
CBDV	0.030	0.015	0.002	0.004	0.014	0.000
CBDVA	0.054	0.027	0.007	0.022	0.013	0.000
CBL	0.0128	0.0064	0.0005	0.0011	0.0063	0.0000
CBLA	0.020	0.010	0.002	0.006	0.001	0.008

Metrological traceability

Results for Δ^9 -THC and CBD presented in this certificate are traceable to the SI through gravimetrically prepared standards of NRC THCN-1 and CBDN-1 respectively, which were employed as the primary calibrators for LC-MS/MS. All other cannabinoids were purchased as certified reference materials from Cerilliant Corporation (Round Rock, Texas, USA) and their purity assignment was verified independently at the NRC. As such, HEMP-1 serves as a suitable reference material for laboratory quality assurance programs, as outlined in ISO/IEC 17025.

Quality Management System (ISO 17034, ISO/IEC 17025)

This material was produced in compliance with the NRC Metrology Quality Management System, which conforms to the requirements of ISO 17034 and ISO/IEC 17025.

Updates

Users should ensure that the certificate they have is current. For updates, please refer to doi.org/10.4224/crm.2021.hemp-1.

References

- [1] McRae, G, Melanson, JE. Quantitative determination and validation of 17 cannabinoids in cannabis and hemp using liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. (2020), 412 (27): 7381-93. <http://doi.org/10.1007/s00216-020-02862-8>
- [2] Evaluation of measurement data: Guide to the expression of uncertainty in measurement JCGM 100:2008. <https://www.bipm.org/en/publications/guides/gum.html>
- [3] Meija J, McRae G, Miles CO, Melanson JE. Thermal stability of cannabinoids in dried cannabis: a kinetic study. Anal Bioanal Chem. (2021). <https://doi.org/10.1007/s00216-020-03098-2>
- [4] van der Veen AMH. Bayesian analysis of homogeneity studies in the production of reference materials. Accred Qual Assur. (2017), 22 (6): 307-319. <http://doi.org/10.1007/s00769-017-1292-6>

Cited by

A list of scientific publications citing HEMP-1 can be found at doi.org/10.4224/crm.2021.hemp-1.

Authorship

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HEMP-1

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Date of expiry: June 2028

Date of revision: April 2024 (certified values updated, information values added, editorial changes, date of expiry extended)

Approved by: _____

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Team Leader, Organic Chemical Metrology
NRC Metrology

This Certificate is only valid if the corresponding material was obtained directly from NRC or an Authorized Reseller.

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