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Certificate of Analysis

Certified Reference Material

CBIS-1

Certified Reference Material of dried, ground cannabis

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

The certified values represent the mass fraction of cannabinoids in CBIS-1 based on results from data generated at the National Research Council of Canada (NRC). Values were assigned using a liquid chromatography – tandem mass spectrometry (LC−MS/MS) method [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 CBIS-1 (k = 2, 95 % CI)

Substance	Symbol	Molecular formula	Mass fraction mg/g
Δ^9-tetrahydrocannabinol (a)	Δ^9-THC	$C_{21}H_{30}O_2$	48.9 ± 6.4
Δ^9-tetrahydrocannabinolic acid (a)	Δ^9-THCA	$C_{22}H_{30}O_4$	124 ± 7
Total Δ^9-tetrahydrocannabinol (a)[1]	Total Δ^9-THC	-	158 ± 9
cannabidiol (a)	CBD	$C_{21}H_{30}O_2$	8.33 ± 0.72
cannabidiolic acid (a)	CBDA	$C_{22}H_{30}O_4$	23.0 ± 1.0
Total cannabidiol (a)[1]	Total CBD	-	28.5 ± 1.0
cannabigerol (a)	CBG	$C_{21}H_{32}O_2$	1.78 ± 0.18
cannabigerolic acid (a)	CBGA	$C_{22}H_{32}O_4$	3.89 ± 0.20
cannabinol (a)	CBN	$C_{21}H_{26}O_2$	3.39 ± 0.42
cannabinolic acid (a)	CBNA	$C_{22}H_{26}O_4$	1.84 ± 0.12
cannabichromene (a)	CBC	$C_{21}H_{30}O_2$	1.02 ± 0.08
cannabichromenic acid (a)	CBCA	$C_{22}H_{30}O_4$	1.67 ± 0.10
tetrahydrocannabivarin (a)	THCV	$C_{19}H_{26}O_2$	0.252 ± 0.026
tetrahydrocannabivarinic acid (a)	THCVA	$C_{20}H_{26}O_4$	0.446 ± 0.034
cannabidivarin (a)	CBDV	$C_{19}H_{26}O_2$	0.0415 ± 0.0038
cannabidivarinic acid (a)	CBDVA	$C_{20}H_{26}O_4$	0.126 ± 0.008

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

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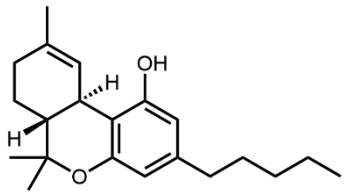
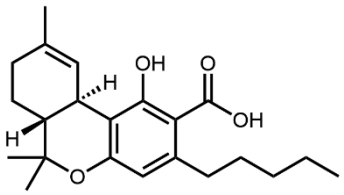
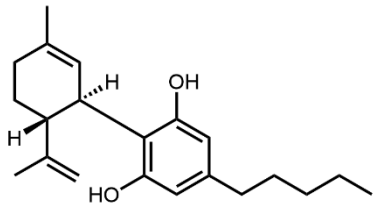


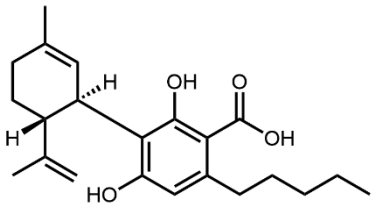
Δ^9-tetrahydrocannabinol (Δ^9-THC)
CAS registry number: 1972-08-3
InChI Key: CYQFCXCEBYINGO-IAGOWNOFSA-N
Molecular formula: $C_{21}H_{30}O_2$
Molar mass: 314.46 ± 0.02 g/mol



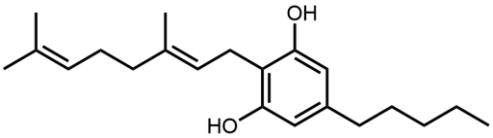
Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA)
CAS registry number: 23978-85-0
InChI Key: UCONUSSAWGCZMV-HZPDHXFCSA-N
Molecular formula: $C_{22}H_{30}O_4$
Molar mass: 358.47 ± 0.02 g/mol



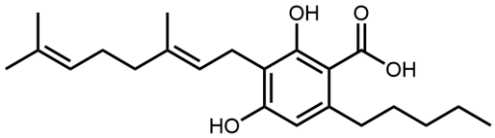
cannabidiol (CBD)
CAS registry number: 13956-29-1
InChI Key: QHMBSVQNZZTUGM-ZWKOTPCHSA-N
Molecular formula: $C_{21}H_{30}O_2$
Molar mass: 314.46 ± 0.02 g/mol



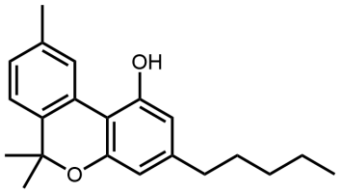
cannabidiolic acid (CBDA)
CAS registry number: 1244-58-2
InChI Key: WVOLTBSCXRRQFR-DLBZAZTESA-N
Molecular formula: $C_{22}H_{30}O_4$
Molar mass: 358.47 ± 0.02 g/mol



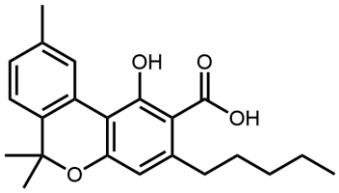
cannabigerol (CBG)
CAS registry number: 25654-31-3
InChI Key: QXACEHWTBCFNSA-SFQUDFHCSA-N
Molecular formula: $C_{21}H_{32}O_2$
Molar mass: 316.48 ± 0.02 g/mol



cannabigerolic acid (CBGA)
CAS registry number: 25555-57-1
InChI Key: SEEZIOZEUUMJME-FOWTUZBSSA-N
Molecular formula: $C_{22}H_{32}O_4$
Molar mass: 360.49 ± 0.02 g/mol



cannabinol (CBN)
CAS registry number: 521-35-7
InChi Key: VBGLYOIFKLUMQG-UHFFFAOYSA-N
Molecular formula: $C_{21}H_{26}O_2$
Molar mass: 310.43 ± 0.02 g/mol



cannabinolic acid (CBNA)
CAS registry number: 2808-39-1
InChi Key: KXKOBIRSQLNUPS-UHFFFAOYSA-N
Molecular formula: $C_{22}H_{26}O_4$
Molar mass: 354.43 ± 0.02 g/mol

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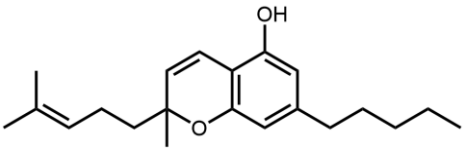
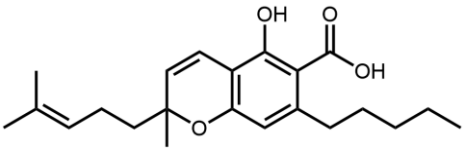
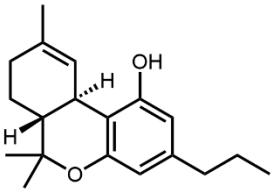


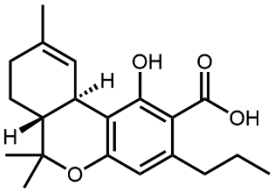
cannabichromene (CBC)
CAS registry number: 20675-51-8
InChI Key: UVOLYTDXHDXWJU-UHFFFAOYSA-N
Molecular formula: $C_{21}H_{30}O_2$
Molar mass: 314.46 ± 0.02 g/mol



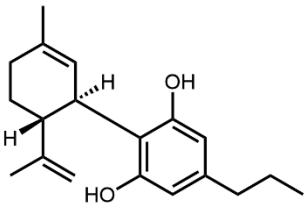
cannabichromenic acid (CBCA)
CAS registry number: 185505-15-1
InChI Key: HRHJHXJQMNWQTF-UHFFFAOYSA-N
Molecular formula: $C_{22}H_{30}O_4$
Molar mass: 358.47 ± 0.02 g/mol



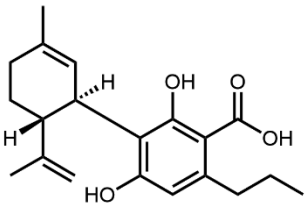
tetrahydrocannabivarin (THCV)
CAS registry number: 31262-37-0
InChI Key: ZROLHBHDLIHEMS-HUUCEWRRSA-N
Molecular formula: $C_{19}H_{26}O_2$
Molar mass: 286.41 ± 0.02 g/mol



tetrahydrocannabivarinic acid (THCVA)
CAS registry number: 39986-26-0
InChI Key: IQSYWEWTWDEVNO-ZIAGYGMSSA-N
Molecular formula: $C_{20}H_{26}O_4$
Molar mass: 330.42 ± 0.02 g/mol



cannabidivarin (CBDV)
CAS registry number: 24274-48-4
InChI Key: REOZWEGFPHTFEI-JKSUJKDBSA-N
Molecular formula: $C_{19}H_{26}O_2$
Molar mass: 286.41 ± 0.02 g/mol



cannabidivarinic acid (CBDVA)
CAS registry number: 31932-13-5
InChI Key: CZXWOKHVLNYAHI-LSDHHAIUSA-N
Molecular formula: $C_{20}H_{26}O_4$
Molar mass: 330.42 ± 0.02 g/mol

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Table 2: Information values for CBIS-1

Element	Mass fraction µg/g
arsenic (b)	0.002
cadmium (b,c)	0.07
chromium (b,c)	2
lead (b,c)	0.5

Coding

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

- **a** Liquid chromatography – tandem mass spectrometry (LC−MS/MS)
- **b** High resolution – inductively coupled plasma – mass spectrometry (HR−ICP−MS)
- **c** Triple quadrupole – inductively coupled plasma – mass spectrometry (3Q−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, and CBDVA in cannabis.

Storage

At a minimum, the material should be stored away from light in a controlled cold temperature environment such as a freezer at –20 °C. For maximum stability, storage at –80 °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. It is recommended to not exceed five freeze-thaw cycles. A minimum sample mass of 200 mg is recommended.

Preparation of material

CBIS-1 was prepared by blending two strains of *Cannabis sativa*, one containing primarily Δ^9-THC and Δ^9-THCA, and the other containing primarily CBD and CBDA. The strains were blended at ambient temperature and passed through a sieve to remove larger particulates. A second blending and sieving was performed to further remove particulates. The material was homogenized by hand mixing and shaking prior to being bottled in 1 g aliquots.

Stability

The effect of freeze-thaw (F/T), transportation, and long-term stability of the cannabinoids in CBIS-1 was assessed at the NRC using LC−MS/MS. Freeze-thaw stability was assessed over five F/T cycles from –20 °C to +20 °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 CBIS-1 were stored at temperatures ranging from –20 °C to +40 °C for up to four years and compared to reference samples stored at –80 °C. Degradation of each cannabinoid was modelled using a network of first-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 °C reference conditions and +20 °C over 2 weeks and at –20 °C over 2 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 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 ($u_{stability}$), and uncertainties related to the different methods (u_{method}). Expressed as standard uncertainties, these components are listed in Table 3. Along with the LC−MS/MS method, a validated liquid chromatography – ultraviolet spectrophotometry (LC−UV) method [5], adapted to cover a greater number of cannabinoids, was employed to obtain u_{method}. The lower relative $U_{k=2}$ values associated with the Δ^9-THC and CBD totals compared to their individual acid and neutral forms is due to the acids decarboxylating to the neutral forms, which masks between-unit variation and uncertainty associated with stability of the totals.

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

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
Δ^9-THC	6.4	3.2	0.5	1.5	2.8	0.0
Δ^9-THCA	7	3.3	1.0	1.6	2.1	1.8

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
Total Δ^9-THC	9	4.6	1.1	1.0	4.2	1.4
CBD	0.72	0.36	0.05	0.30	0.19	0.04
CBDA	1.0	0.5	0.1	0.4	0.3	0.00
Total CBD	1.0	0.5	0.1	0.4	0.2	0.00
CBG	0.18	0.09	0.01	0.03	0.08	0.02
CBGA	0.20	0.10	0.03	0.04	0.06	0.07
CBN	0.42	0.21	0.02	0.13	0.16	0.05
CBNA	0.12	0.06	0.01	0.03	0.05	0.00
CBC	0.08	0.04	0.01	0.03	0.03	0.01
CBCA	0.10	0.05	0.01	0.03	0.03	0.00
THCV	0.026	0.013	0.004	0.006	0.011	0.000
THCVA	0.034	0.017	0.004	0.010	0.007	0.012
CBDV	0.0038	0.0019	0.0007	0.0013	0.0011	0.0006
CBDVA	0.008	0.004	0.002	0.002	0.002	0.000

Metrological traceability

Results for CBD presented in this certificate are traceable to the SI through gravimetrically prepared standards of NIST SRM 350b (benzoic acid) employed as an internal standard for quantitative proton nuclear magnetic resonance (^{1}H-qNMR). 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, CBIS-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.cbis-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

[5] Mudge, EM, Brown, PN. Determination of cannabinoids in cannabis sativa dried flowers and oils by LC-UV: Single-laboratory validation, First action 2018.10. J AOAC Int. (2019), 103 (2): 489-493. http://doi.org/10.5740/jaoacint.19-0197

Cited by

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

Authorship

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CBIS-1

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Date of revision: April 2024 (editorial changes, date of expiry extended)

Approved by:

Andreas Brinkmann, Ph.D.
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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