Designation: ~~D8375 – 22~~ D8375 – 23

Standard Test Method for Determination of Cannabinoid Concentration in Dried Cannabis and Hemp Raw Materials using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)[1]

This standard is issued under the fixed designation D8375; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method allows for the concentration determination of the cannabinoids listed in Table 1, and shall apply to any dried raw material from a cannabis plant (Note 1, Note 2) regardless of the type of cannabis plant from which it was derived.[2] For the sake of brevity, the term "cannabis" shall be used from now on to refer to any type of cannabis plant including those that can be classified as hemp. The procedure includes sub-sampling a ground, homogeneous sample, extraction with methanol:water (80:20, v:v),[3,4] dilution in methanol and analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). The method allows for a wide-range of sample concentrations to be determined by using a 1000-fold calibration range and the option to perform multiple levels of sample dilution. The calibration curve is prepared in methanol over a range of 10 ng⁄mL to 10 000 ng/mL for all seventeen cannabinoids, or a subset of cannabinoids if desired, while the sample extracts are diluted in methanol into the calibration range.[3,4,5] For example, a 1/500 dilution of sample extracts allows concentration determination over a range of 0.5 mg⁄g to 500 mg⁄g in cannabis. The method was validated with quality control samples prepared in methanol, a candidate certified reference material (CRM), and repeat extraction and analysis of cannabinoid samples.[3]

NOTE 1—For this test method, dried raw material from a cannabis plant includes one or more of inflorescence, leaves, or stems.

NOTE 2—Certain jurisdictions or regulations may require specific parts of the plant to be included or excluded for analysis and those regulations will take precedence for the selection of plant parts.

1.2 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *List of Measurable Analytes*—See Table 1.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

[1] This test method is under the jurisdiction of ASTM Committee D37 on Cannabis and is the direct responsibility of Subcommittee D37.03 on Laboratory.

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[2] Health Canada, Guidance Document: Good production practices guide for cannabis Testing for Phytocannabinoids.

[3] McRae, G. and Melanson, J. E., Quantitative determination and validation of 17 cannabinoids in cannabis and hemp using liquid chromatography-tandem mass spectrometry, *Anal Bioanal Chem*, Vol 412, No. 27, 2020, pp. 7381–7393, doi:10.1007/s00216-020-02862-8.

[4] Mudge, E. M., Murch, S. J., Brown, P. N., Leaner and greener analysis of cannabinoids, *Anal Bioanal Chem*, Vol 409, No. 12, 2017, pp. 3153–3163, doi: 10.1007/s00216-017-0256-3.

[5] Vaclavik, L., Benes, F., Fenclova, M., Hricko, J., Krmela, A., Svobodova, V., et al. Quantitation of cannabinoids in cannabis dried plant materials, concentrates, and oils using liquid chromatography-diode array detection technique with optional mass spectrometric detection: single-laboratory validation study, first action 2018.11, *J AOAC Int*. Vol 102, No. 6, 2019, pp. 1822–33

TABLE 1 List of Measurable Analytes

Analyte Name	Analyte Abbreviation
delta-9-tetrahydrocannabinol	Δ^9-THC
delta-9-tetrahydrocannabinolic acid	Δ^9-THCA
cannabidiol	CBD
cannabidiolic acid	CBDA
cannabigerol	CBG
cannabigerolic acid	CBGA
cannabigerovarin	CBGV
cannabigerovarinic acid	CBGVA
cannabinol	CBN
cannabinolic acid	CBNA
cannabivarin	CBV
cannabichromene	CBC
cannabichromenic acid	CBCA
tetrahydrocannibivarin	THCV
tetrahydrocannibivarinic acid	THCVA
cannibidivarin	CBDV
cannibidivarinic acid	CBDVA
cannabicyclol	CBL
cannabicyclolic acid	CBLA
delta-8 tetrahydrocannabinol	Δ^8-THC

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[6]

D1193 Specification for Reagent Water
D8245 Guide for Disposal of Resin-Containing Cannabis Raw Materials and Downstream Products
D8270 Terminology Relating to Cannabis
D8282 Practice for Laboratory Test Method Validation and Method Development
E203 Test Method for Water Using Volumetric Karl Fischer Titration

3. Terminology

3.1 *Definitions*—For general terms related to cannabis, refer to Terminology D8270.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *blank, n*—a reagent only sample extracted and processed under the same conditions as cannabis samples without the addition of internal standard working solution (ISWS).

3.2.2 *blank-0, n*—a reagent only sample extracted and processed under the same conditions as cannabis samples with the addition of ISWS.

3.3 *Abbreviations:*

3.3.1 *Conc.*—concentration

3.3.2 *LOD*—limit of detection

3.3.3 *LOQ*—limit of quantitation

3.3.4 *RSD*—relative standard deviation

3.3.5 *Vol.*—volume

[6] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

4. Summary of Test Method

4.1 The quantitative analysis of cannabinoids in cannabis is accomplished by extraction of ground plant material with methanol:water (80:20, v:v), followed by dilution in methanol and analysis using LC-MS/MS.

4.2 Cannabinoids are identified by retention time and by selective reaction monitoring (SRM) transitions. An SRM transition consists of a pseudo-molecular ion, selected in quadrupole one, and a product ion, selected in quadrupole three. Pseudo-molecular ions are fragmented to product ions in quadrupole two (collision cell). The product ion selected in quadrupole three is transmitted to the detector of the mass spectrometer to produce a signal, resulting in a peak for the cannabinoid in the chromatogram. Cannabinoids are quantitated using the designated quantitative SRM transition. The final result reported for each sample lists the concentration of cannabinoids in cannabis.

5. Significance and Use

5.1 The analysis and reporting of cannabinoid content in cannabis and hemp is required to address human health and safety concerns, satisfy testing and labeling requirements, and meet the regulatory guidelines of various jurisdictions. This test method is useful in providing quantitative results for up to seventeen cannabinoids in dried cannabis and hemp raw material samples.

6. Interferences

6.1 Contaminants in solvents, reagents, glassware, and other apparatus producing discrete artifacts or elevated baselines have the potential to cause method interferences. All of these materials are demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as samples. A blank sample is used to evaluate potential interferences for the internal standards while a blank-0 sample is used to evaluate interferences for the analytes.

6.2 Contaminants that are co-extracted from the sample have the potential to cause method interferences. The extent of matrix interferences can vary considerably from sample source depending on variations of the sample matrix.

7. Apparatus

7.1 *Analytical Balance*—Any analytical balance capable of readability down to 0.1 mg.

7.2 *Grinder/Homogenizer*—Any grinder capable of grinding dried cannabis raw materials to a powder form.

7.3 *Solvent Dispenser*—Any solvent dispenser capable of dispensing 5 mL ± 0.1 mL.

7.4 *Multi-tube Vortex Mixer*—Any vortexer capable of vortex mixing multiple 15 mL tubes at high speed.

7.5 *Centrifuge*—Any centrifuge capable of holding 15 mL tubes and operating at 5000 r⁄min ± 500 r⁄min (4700 RCF ± 470 RCF).

7.6 *LC-MS/MS System:*

7.6.1 *Liquid Chromatography (LC) System*—A complete LC system, including pump, temperature controlled autosampler, and column heater is required in order to analyze samples. Any LC system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the standard shall be considered suitable for use.

7.6.2 *Tandem Mass Spectrometer (MS/MS) System*—A MS/MS system capable of selective reactive monitoring (SRM) analysis shall be considered suitable for use.

7.6.3 *Analytical Column*—Any column (Note 3) that achieves peak resolution ≥ 1 for cannabinoids having the same mass ± 2 *m/z* may be used. The retention times and order of elution may change depending on the column used and need to be monitored.

NOTE 3—A reverse-phase analytical column ~~(C18-Amide, 100~~(C18, 150 × 2.1 mm, ~~3 µm)~~ 2.6 µm) with an analytical guard column ~~(C18-Amide,~~(C18, 10 × 2.1 mm, ~~3 µm)~~ 2.6 µm) was used to develop this test method. While not required, use of a guard column is recommended to extend the life of the analytical column.

8. Reagents and Materials

8.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[7] Other grades may be used, provided it is pure enough to be used without lessening the accuracy of the determination.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type I of Specification D1193.

8.3 *Acetonitrile,* LC-MS grade, or equivalent.

8.4 *Cannabinoid reference standard solutions,* CRM or equivalent.

8.4.1 Cannabinoid reference standard solutions are commercially available individually or as mixed standards, typically at concentrations of 1.0 mg/mL or 0.5 mg/mL in methanol or acetonitrile.

8.5 Cannabinoid internal standard solutions-isotopically labeled: THC-d3, ~~CBD-d3 and CBN-d3.~~THCA-d3, CBD-d3, CBG-d3, CBGA-d3, CBN-d3, and CBCA-d3. CRM or equivalent.

8.5.1 Isotopically-labeled cannabinoid internal standard solutions are commercially available, typically at concentrations of 0.1 mg/mL in methanol or acetonitrile.

8.6 *Cannabis CRM,* if available.

8.7 *Formic acid,* LC-MS grade, or equivalent.

8.8 *Hemp CRM,* if available.

8.9 *Methanol,* LC-MS grade, or equivalent.

9. Hazards

9.1 All work with solvents shall be carried out in a fume hood while personal protection equipment is worn, including gloves, safety glasses or goggles, and a lab coat.

9.2 Several solvents are used in this test method, including methanol and acetonitrile. Check their safety data sheet to identify specific hazards. Follow local regulations for proper disposal of spent chemicals (see Guide D8245).

10. Calibration and Standardization

10.1 The mass spectrometer shall be calibrated per manufacturer specifications before analysis. In order to obtain valid and accurate analytical values within the confidence limits, the following procedures shall be followed when performing the test method.

10.2 *Calibration and Standardization:*

10.2.1 Seven (7) calibration standards (CAL) levels and one (1) independent check sample (ICS) level shall be prepared, with each containing up to ~~seventeen (17)~~twenty (20) cannabinoids. Prepare a minimum of two (2) master calibration standard (MCS) solutions by combining the components in Table 2, or equivalent, and mixing well. The two MCS solutions shall be prepared using reference standard solutions from different suppliers, different lots, or different vials/ampules. One MCS solution (MCS-1) is to be used for preparation of the CAL solutions and the other (MCS-2) for preparation of the ICS solution. MCS solutions with fewer

[7] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the United States *Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

TABLE 2 MCS Solution Preparation

Reference Standard Solution/Solvent	Cannabinoid Conc. (µg/mL)	Reference Standard Solution Vol. (µL)	Conc. in mixture (µg/mL)
~~Δ^9-THCA~~	~~1000~~	~~500~~	~~50.0~~
Δ^9-THCA	1000	400	40.0
~~CBDA~~	~~1000~~	~~500~~	~~50.0~~
CBDA	1000	400	40.0
~~CBGA~~	~~1000~~	~~500~~	~~50.0~~
CBGA	1000	400	40.0
CBGVA	1000	400	40.0
~~CBNA~~	~~1000~~	~~500~~	~~50.0~~
CBNA	1000	400	40.0
~~THCVA~~	~~1000~~	~~500~~	~~50.0~~
THCVA	1000	400	40.0
~~CBCA~~	~~1000~~	~~500~~	~~50.0~~
CBCA	1000	400	40.0
~~CBDVA~~	~~1000~~	~~500~~	~~50.0~~
CBDVA	1000	400	40.0
~~CBLA~~	~~500~~	~~1000~~	~~50.0~~
CBLA	500	800	40.0
~~Δ^9-THC~~	~~1000~~	~~500~~	~~50.0~~
Δ^9-THC	1000	400	40.0
~~CBD~~	~~1000~~	~~500~~	~~50.0~~
CBD	1000	400	40.0
~~CBG~~	~~1000~~	~~500~~	~~50.0~~
CBG	1000	400	40.0
CBGV	1000	400	40.0
~~CBN~~	~~1000~~	~~500~~	~~50.0~~
CBN	1000	400	40.0
CBV	1000	400	40.0
~~THCV~~	~~1000~~	~~500~~	~~50.0~~
THCV	1000	400	40.0
~~CBC~~	~~1000~~	~~500~~	~~50.0~~
CBC	1000	400	40.0
~~CBDV~~	~~1000~~	~~500~~	~~50.0~~
CBDV	1000	400	40.0
~~CBL~~	~~1000~~	~~500~~	~~50.0~~
CBL	1000	400	40.0
~~Δ^8-THC~~	~~1000~~	~~500~~	~~50.0~~
Δ^8-THC	1000	400	40.0
~~MeOH~~	-	~~1000~~	-
MeOH	-	1600	-
Total Volume	-	10 000	-

cannabinoids may be prepared provided that the analyte concentrations remain the same. Cannabinoid reference standard solutions and CRMs shall be stored according to the manufacturers' instructions and used by the expiration date stated by the manufacturer. MCS solutions, CAL solutions, and ICS solutions shall be stored at –20 °C or lower and replaced every three months.

10.2.1.1 Commercial suppliers may supply cannabinoid reference standard solutions at different concentrations or as a mix of multiple cannabinoids. Those reference standard solutions may be used to prepare the MCS solutions provided the volumes in Table 2 are adjusted accordingly and the final cannabinoid concentrations in the MCS solutions remain the same.

10.2.2 Preparation of CAL and ICS solutions in methanol is performed as shown in Table 3.

10.2.3 Preparation of internal standard working solution (ISWS) in methanol is performed as shown in Table 4.

10.2.4 *Routine Recovery*—Routine recovery shall be demonstrated in each sample analysis batch by processing a cannabis or hemp matrix CRM (10.2.5) or by preparing and processing a routine recovery spike (RRS) (10.2.6).

10.2.4.1 Analysis of a cannabis or hemp matrix CRM is the preferable option to provide evidence of method recovery.

10.2.5 Routine recovery using a cannabis or hemp matrix CRM: Cannabis or hemp matrix CRMs may be purchased from commercial suppliers and shall include a valid certificate of analysis.

10.2.5.1 A minimum of one (1) matrix CRM sample shall be taken through the complete analytical test method procedure. The

TABLE 3 CAL Solution and ICS Solution Preparation

NOTE 1—Final volume may be changed provided the proportions remain the same.

CAL/ICS Solution	Solution Used	Vol. of Solution (µL)	Vol. of MeOH (µL)	Final Vol. (µL)	Conc. (ng/mL)
~~CAL-7~~	~~MCS-1~~	~~400~~	~~1600~~	~~2000~~	~~10 000~~
CAL-7	MCS-1	500	1500	2000	10 000
~~CAL-6~~	~~MCS-1~~	~~360~~	~~1640~~	~~2000~~	~~9000~~
CAL-6	MCS-1	450	1550	2000	9000
~~CAL-5~~	~~MCS-1~~	~~240~~	~~1760~~	~~2000~~	~~6000~~
CAL-5	MCS-1	300	1700	2000	6000
CAL-4	CAL-7	200	1800	2000	1000
CAL-3	CAL-4	200	1800	2000	100
CAL-2	CAL-3	400	1600	2000	20
CAL-1	CAL-3	200	1800	2000	10
~~ICS-1~~	~~MCS-2~~	~~120~~	~~3880~~	~~4000~~	~~1500~~
ICS-1	MCS-2	150	3850	4000	1500

TABLE 4 ISWS Preparation

NOTE 1—Final volume may be changed provided the ~~proportions~~concentrations remain the same. Internal standards may be omitted if the corresponding cannabinoid analyte is not included in the MCS.

Cannabinoid Stock Solution/ Solvent	Stock Conc (µg/mL)	Stock Vol. (µL)	Conc. in mixture (ng/mL)
THC-d3	100	250	500
THCA-d3	100	250	500
CBD-d3	100	250	500
CBG-d3	100	250	500
CBGA-d3	100	250	500
CBN-d3	100	250	500
CBCA-d3	100	250	500
~~MeOH~~	-	~~49 250~~	-
MeOH	-	48 250	-
Total volume	-	50 000	-

calculated concentrations of each analyte included in the CRM certificate of analysis shall have percent bias ≤20 % or ≤ the expanded uncertainty reported in the certificate of analysis, whichever is greater.

10.2.6 *Routine recovery using a RRS:*

10.2.6.1 RRS samples shall be prepared in homogeneous, ground cannabis samples that have known cannabinoid concentrations. It is recommended to spike a minimum of five (5) cannabinoids into the sample, while a minimum of two (2) cannabinoids shall be used to calculate routine recovery. Cannabinoids used to calculate routine recovery shall have a post-spike matrix concentration level ≥ two (2) times the level present in the un-spiked cannabis sample and provide a concentration ≥ three (3) times the CAL-1 concentration after sample extraction and dilution.

10.2.6.2 A minimum of one (1) RRS and one (1) un-spiked matrix sample shall be taken through the complete analytical test method procedure. The recovery shall be calculated using blank subtraction as shown in Eq 1 and shall be between 80 % and 120 %.

$$\text{Recovery} = (100)(\text{Crrs} - \text{Cucs})/\text{Csa} \quad (1)$$

where:

Recovery = recovery of spiked cannabinoid from the cannabis sample in %,
Crrs = concentration of cannabinoid in the RRS after spiking,
Cucs = concentration of cannabinoid in the un-spiked cannabis sample, and
Csa = concentration, after addition, of cannabinoid spiked into the cannabis sample.

10.2.6.3 Cannabis reference standard solutions may be used to spike the RRS samples. It is recommended to spike as many

cannabinoids as possible during preparation of the RRS, however this will be limited due to reference standard solution concentrations, volumes and cannabinoid concentrations in the un-spiked cannabis samples.

10.2.7 Inject each CAL to obtain the chromatograms, monitoring the SRM transitions of each analyte and its internal standard. Calibration software is used to conduct quantitation of the target analytes with SRM transitions of each analyte used for quantitation and confirmation.

10.2.8 The calibration software manual should be consulted to use the software properly. The quantitative method uses peak area ratios of the analyte/internal standard vs the analyte concentration in units of ng/mL. Regressions (that is, linear or quadratic depending on the instrument used) may be generated using the data system software. Forcing the regression line through the origin is not recommended. Each CAL used to generate the regression shall have a calculated concentration ≤15 % bias (≤20 % bias for CAL-1) from the nominal concentration and shall be rejected if this specification is not met. Certain jurisdictions or regulations may require more stringent specifications and those regulations will take precedence.

10.2.9 Linear calibration may be used if the coefficient of determination, r^2, is ≥ 0.99. A weighting of 1/x or $1/x^2$ is recommended to give more emphasis to the lower concentrations. A minimum of five (5) points is considered acceptable for each analyte. Rejected CALs shall not be adjacent to one another. If the low or high CAL point are rejected, the reporting range shall be modified to reflect this change (Note 4).

10.2.10 Quadratic calibration may be used if the coefficient of determination, r^2, is ≥ 0.99. A weighting of 1/x or $1/x^2$ is recommended to give more emphasis to the lower concentrations. A minimum of five (5) points is considered acceptable for each analyte. Rejected CALs shall not be adjacent to one another. If the low or high CAL point are rejected, the reporting range shall be modified to reflect this change (Note 4).

NOTE 4—Certain jurisdictions or regulations may prohibit the rejection of the high or low calibration points and those regulations will take precedence.

10.2.11 The retention time window of the SRM transitions shall be within ±5 % of the retention time of the analyte in a mid-point CAL. If this is not the case, re-examine the CAL to determine if there was a shift in retention time during the analysis. If a retention time shift occurred, the sample shall be re-injected. If the retention time is still incorrect in the sample, refer to the peak as an unknown.

10.2.12 *ICS*—Inject a minimum of one (1) ICS at the beginning of each batch. The concentration of the ICS shall have a bias ≤15 % of the nominal concentration.

10.2.13 *Continuing Calibration Verification (CCV)*—Inject an ICS or mid-level CAL at the beginning, middle and end of each batch, including injections at a minimum of every 10 samples. The concentration of the ICS or CAL shall have a bias ≤15 % of the nominal concentration. If this is not the case, any samples injected after the last ICS or CAL that met these specifications shall be re-analyzed. Certain jurisdictions or regulations may require more stringent acceptance specifications and those regulations will take precedence.

10.3 *Method Blanks:*

10.3.1 A blank sample shall be injected at least once in the run. Any peak in the blank sample at the retention time and SRM transitions of the internal standards shall have a peak area ≤5 % of the average of the internal standard peak areas of the CAL samples.

10.3.2 A blank-0 sample shall be injected at the beginning, middle and end of the run, including a blank sample injected a minimum of every 10 samples. Any peak in the blank-0 sample at the retention time and SRM transition of the analytes shall have a concentration ≤20 % of CAL-1 concentration.

10.4 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example: a new analyst or new equipment, perform a precision and bias study to demonstrate the laboratory capability.

10.4.1 If a cannabis or hemp matrix CRM is available, analyze at least four (4) replicates of the CRM. The sample shall be taken through the complete analytical test method. Calculate the mean (average) concentration and % RSD and compare to the concentration in the CRM certificate of analysis. The calculated concentrations of the analytes shall have percent bias ≤15 % or ≤ the expanded uncertainty reported in the certificate of analysis, whichever is greater, and an RSD ≤15 %.

10.4.2 If a cannabis or hemp CRM is not available, the ICS, RRS or an in-house cannabis or hemp reference sample may be used to demonstrate precision and bias.

10.4.3 This study shall be repeated until the single operator precision and bias are within the specifications.

11. Conditioning and Instrument Parameters

11.1 Analyze using a tandem mass spectrometer (MS/MS) coupled to a high-performance liquid chromatography (HPLC) system

11.2 Introduce sample using an autosampler and achieve analyte separation on an appropriate reverse-phase column (Note 5). Equilibrate the instrument by injecting a minimum of one blank sample and one CAL-1 sample to verify analyte retention times and that signal to noise ratios (S/N) of all analytes are ≥10. See Tables 5-8 for additional instrument parameters. Parameters in Table 7 are an example only and may be different in name, number and setting for various instruments. Parameters should be optimized for specific LC-MS/MS systems. Collision energy settings in Table 8 may require optimization for specific mass spectrometers.

NOTE 5—A ~~C18-Amide, 3 µm,~~ C18, 2.6 µm, 2.1 mm × ~~100 mm~~ 150 mm HPLC column fitted with a ~~C18-amide, 3 µm,~~ C18, 2.6 µm, 2.1 mm × 10 mm guard column was used with the gradient described in Table 6 to develop this test method.

11.3 Table 8 illustrates the SRM transitions used for cannabinoids. Bold entries indicate transitions used for quantitation, while non-bold entries indicate transitions used for qualification.

12. Procedure

12.1 Record all sample information in conformance within the requirements of the existing lab management practices as defined within your quality management system (QMS).

12.2 Homogenize the dried cannabis at low temperature using a grinder.

12.3 Weigh 100 mg ± 5 mg of sample into 15 mL tubes, recording the mass to an accuracy of 0.1 mg.

12.4 Add 5 mL ± 0.1 mL of methanol:water (80:20, v:v).

12.4.1 For RRS samples, reduce the volume of methanol:water (80:20, v:v) by the volume of reference standard solutions spiked into the sample.

12.5 Vortex at high speed for 90 s ± 10 s.

TABLE 5 HPLC Conditions

NOTE 1—Parameters may be optimized for specific instruments and analytical column used.

Parameter	Setting
Column	reverse phase
Guard Column	reverse phase
Mobile Phase A	water:formic acid (100:0.1, v:v)
Mobile Phase B	acetonitrile:formic acid (100:0.1, v:v)
Flow Rate (mL/min)	0.5
~~Run Time (min)~~	~~21~~
Run Time (min)	18
Column temperature (°C)	40
~~Switch Valve times (min)~~	~~0-4.0 min to waste, 4.0-17.0 min to MS, 17.0-19.0 min to waste~~
Switch Valve times (min)	0-1.5 min to waste, 1.5-14.0 min to MS, 14.0-18.0 min to waste
Injection Volume (µL)	1.0
Needle Wash	acetonitrile:methanol:water:formic acid (40:40:20:1, v:v:v:v)
~~Autosampler Temperature (°C)~~	~~5°C~~
Autosampler Temperature (°C)	5 °C

TABLE 6 HPLC Gradient

NOTE 1—Gradient may be optimized for specific columns used.

Time (min)	Flow (mL/min)	%B
~~0.0~~	~~0.5~~	~~57~~
~~5.0~~	~~0.5~~	~~70~~
~~11.0~~	~~0.5~~	~~75~~
~~13.0~~	~~0.5~~	~~80~~
~~14.0~~	~~0.5~~	~~95~~
~~17.0~~	~~0.5~~	~~98~~
~~17.2~~	~~0.5~~	~~57~~
~~19.0~~	~~0.5~~	~~57~~

TABLE 6 HPLC Gradient

NOTE 1—Gradient may be optimized for specific columns used.

Time (min)	Flow (mL/min)	%B
0.0	0.5	60
8.0	0.5	68
13.5	0.5	68
13.6	0.5	95
14.5	0.5	95
14.6	0.5	60
18.0	0.5	60

TABLE 7 Mass Spectrometer Parameters

NOTE 1—Parameters may be optimized for specific instruments used.

Parameter	Setting
Scan Type	SRM
Ion Source	Heated Electrospray
Polarity	Positive
Ion Spray Voltage (V)	4000
Sheath Gas (arbitrary units)	50
Aux Gas (arbitrary units)	20
Sweep Gas (arbitrary units)	2
Ion Transfer Tube Temperature (°C)	325
Vaporizer Temperature (°C)	150
~~Collision Gas (mTorr)~~	~~1.5~~
Collision Gas (Pa)	0.2
Dwell Time (msec)	40

TABLE 8 SRM Transitions for Cannabinoids

NOTE 1—Retention times will vary with column and mobile phase used.

NOTE 2—Collision energy may be optimized for specific instruments used.

Compound	Retention Time (min)	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)
CBDVA	2.2	**313**	**191**	**26**
		313	233	20
~~CBDV~~	~~5.4~~	~~**287**~~	~~**165**~~	~~**23**~~
CBDV	2.6	**287**	**165**	**23**
		287	123	30
~~THCV~~	~~6.5~~	~~**287**~~	~~**165**~~	~~**23**~~
CBGVA	2.6	**315**	**191**	**23**
		~~287~~	~~123~~	~~30~~
		333	191	26
~~CBDVA~~	~~7.2~~	~~**313**~~	~~**191**~~	~~**26**~~
CBV	3.5	**283**	**223**	**20**
		~~313~~	~~233~~	~~20~~
		283	265	16
CBDA	3.7	**341**	**219**	**26**
		359	219	25
~~CBD~~	~~7.9~~	~~**315**~~	~~**193**~~	~~**21**~~
CBGA	4.0	**343**	**219**	**23**
		~~315~~	~~135~~	~~20~~
		361	219	26
~~CBG~~	~~9.1~~	~~**317**~~	~~**193**~~	~~**16**~~
CBG	4.4	**317**	**193**	**16**
		317	123	32
~~CBN~~	~~9.2~~	~~**311**~~	~~**223**~~	~~**22**~~
THCV	4.4	**287**	**165**	**23**
		~~311~~	~~241~~	~~18~~
		287	123	30
CBD	4.6	**315**	**193**	**21**
		315	135	20
~~THCVA~~	~~9.3~~	~~**313**~~	~~**191**~~	~~**26**~~
THCVA	5.6	**313**	**191**	**26**
		313	233	20
~~THC~~	~~9.8~~	~~**315**~~	~~**193**~~	~~**21**~~
CBN	6.5	**311**	**223**	**22**
		~~315~~	~~135~~	~~20~~
		311	241	18
~~Δ8-THC~~	~~10.3~~	~~**315**~~	~~**193**~~	~~**21**~~
CBNA	7.8	**337**	**235**	**25**
		~~315~~	~~135~~	~~20~~
		337	253	23
~~CBC~~	~~10.8~~	~~**315**~~	~~**193**~~	~~**21**~~
Δ9-THC	7.8	**315**	**193**	**21**
		315	135	20
~~CBDA~~	~~11.0~~	~~**341**~~	~~**219**~~	~~**26**~~
Δ8-THC	8.3	**315**	**193**	**21**
		~~359~~	~~219~~	~~25~~
		315	135	20
~~CBL~~	~~11.6~~	~~**315**~~	~~**235**~~	~~**18**~~
CBL	9.1	**315**	**235**	**18**
		315	81	30
~~CBNA~~	~~12.9~~	~~**337**~~	~~**235**~~	~~**25**~~
		~~337~~	~~253~~	~~23~~
~~THCA~~	~~13.4~~	~~**341**~~	~~**219**~~	~~**26**~~
THCA	9.6	**341**	**219**	**26**
		359	219	25
~~CBGA~~	~~13.6~~	~~**343**~~	~~**219**~~	~~**23**~~
CBC	10.1	**315**	**193**	**21**
		315	259	14
CBLA	11.2	**359**	**261**	**25**
		~~361~~	~~219~~	~~26~~
		359	219	32
~~CBCA~~	~~14.1~~	~~**341**~~	~~**219**~~	~~**26**~~
CBCA	11.4	**341**	**219**	**26**
		359	219	25
~~CBLA~~	~~14.5~~	~~359~~	~~261~~	~~25~~
CBGA-d3	4.0	346	222	23
		~~359~~	~~219~~	~~32~~
CBG-d3	4.4	320	196	16
~~CBD-d3~~	~~7.9~~	~~318~~	~~196~~	~~21~~

Compound	Retention Time (min)	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)
CBD-d3	4.5	318	196	21
~~CBN-d3~~	~~9.2~~	~~314~~	~~223~~	~~21~~
CBN-d3	6.4	314	223	21
~~THC-d3~~	~~9.8~~	~~318~~	~~196~~	~~22~~
THC-d3	7.8	318	196	22
THCA-d3	9.6	344	222	26
CBCA-d3	11.4	362	222	25

QC Sample	QC-LLOQ (ng/mL)	QC-1 (ng/mL)	QC-2 (ng/mL)
	10	30	1500
Precision (%)	1.0 %	0.7 %	2.2 %
Accuracy (%)	99.4 %	94.1 %	97.9 %
QC sample Inter-Batch Stats	QC-LLOQ	QC-1	QC-2
n	12	12	12
Av.	10.2	28.1	1477
Precision (%)	3.0 %	2.7 %	1.7 %
Accuracy (%)	102.2 %	93.7 %	98.5 %

TABLE 9 Δ9-THC Precision, Accuracy, and Recovery

QC Sample	QC-LLOQ (ng/mL)	QC-1 (ng/mL)	QC-2 (ng/mL)	QC-3 (ng/mL)	Cannabis CRM (mg/g)
	10	30	1500	8000	60.3
Batch 1					
Rep-1	10.16	27.9	1454	7954	57.1
Rep-2	10.46	27.9	1471	7968	ALQ
Rep-3	10.09	28.9	1462	7920	ALQ
Rep-4	10.23	28.5	1455	8062	-
Av.	10.235	28.31	1460.6	7975.9	57.1
Precision (%)	1.6 %	1.9 %	0.5 %	0.8 %	N/AP
Accuracy (%)	102.4 %	94.4 %	97.4 %	99.7 %	94.7 %
Batch 2					
Rep-1	10.72	29.0	1466	8007	55.8
Rep-2	10.44	28.7	1471	7884	55.9
Rep-3	10.62	29.2	1482	7965	55.7
Rep-4	9.99	28.5	1466	7905	-
Av.	10.442	28.84	1471.3	7940.1	55.8
Precision (%)	3.1 %	1.1 %	0.5 %	0.7 %	0.2 %
Accuracy (%)	104.4 %	96.1 %	98.1 %	99.3 %	92.5 %
Batch 3					
Rep-1	10.50	29.3	1448	7869	60.2
Rep-2	9.93	29.1	1445	7868	60.7
Rep-3	10.14	29.0	1474	7747	58.5
Rep-4	9.79	29.0	1456	7890	59.3
Av.	10.090	29.10	1456.1	7843.2	59.7
Precision (%)	3.0 %	0.5 %	0.9 %	0.8 %	1.6 %
Accuracy (%)	100.9 %	97.0 %	97.1 %	98.0 %	98.9 %
QC sample Inter-Batch Stats	QC-LLOQ	QC-1	QC-2	QC-3	Cannabis CRM
n	12	12	12	12	8
Av.	10.3	28.7	1463	7920	57.9
Precision (%)	2.8 %	1.7 %	0.8 %	1.0 %	3.5 %
Accuracy (%)	102.6 %	95.8 %	97.5 %	99.0 %	96.0 %

TABLE 10 Δ9-THCA Precision, Accuracy, and Recovery

QC Sample	QC-LLOQ (ng/mL)	QC-1 (ng/mL)	QC-2 (ng/mL)	QC-3 (ng/mL)	Cannabis CRM (mg/g)
	10	30	1500	8000	124
Batch 1					
Rep-1	10.31	29.4	1450	7949	115
Rep-2	10.75	29.2	1486	7803	ALQ
Rep-3	10.13	28.8	1487	7894	ALQ
Rep-4	10.69	27.7	1446	7853	-
Av.	10.474	28.78	1467.2	7874.5	115
Precision (%)	2.9 %	2.7 %	1.5 %	0.8 %	N/AP
Accuracy (%)	104.7 %	95.9 %	97.8 %	98.4 %	92.4 %
Batch 2					
Rep-1	10.21	27.6	1501	7919	107
Rep-2	10.59	27.5	1505	7766	106
Rep-3	10.11	27.3	1476	7793	107
Rep-4	10.13	26.9	1497	7664	-
Av.	10.259	27.32	1494.6	7785.3	107
Precision (%)	2.2 %	1.1 %	0.9 %	1.3 %	0.7 %
Accuracy (%)	102.6 %	91.1 %	99.6 %	97.3 %	86.1 %
Batch 3					
Rep-1	10.03	28.3	1495	7817	117
Rep-2	9.83	28.0	1439	7575	119
Rep-3	10.02	28.2	1443	7619	114
Rep-4	9.87	28.5	1497	7898	116
Av.	9.937	28.24	1468.5	7727.5	117

TABLE 11 CBD Precision, Accuracy, and Recovery

QC Sample	QC-LLOQ (ng/mL)	QC-1 (ng/mL)	QC-2 (ng/mL)
	10	30	1500
Batch 1			
Rep-1	10.57	27.7	1456
Rep-2	10.34	28.3	1476
Rep-3	10.19	28.2	1473
Rep-4	9.96	28.1	1478
Av.	10.263	28.09	1470.7
Precision (%)	2.5 %	1.0 %	0.7 %
Accuracy (%)	102.6 %	93.6 %	98.0 %
Batch 2			
Rep-1	10.86	28.9	1461
Rep-2	10.87	29.0	1462
Rep-3	10.37	28.7	1481
Rep-4	10.62	28.3	1471
Av.	10.677	28.72	1468.7
Precision (%)	2.2 %	1.2 %	0.6 %
Accuracy (%)	106.8 %	95.7 %	97.9 %
Batch 3			
Rep-1	10.35	28.8	1459
Rep-2	10.35	29.2	1466
Rep-3	10.33	29.6	1433
Rep-4	10.39	29.7	1450
Av.	10.355	29.34	1452.0
Precision (%)	0.3 %	1.4 %	1.0 %
Accuracy (%)	103.5 %	97.8 %	96.8 %
QC sample Inter-Batch Stats	QC-LLOQ	QC-1	QC-2
n	12	12	12
Av.	10.4	28.7	1464
Precision (%)	2.5 %	2.2 %	0.9 %
Accuracy (%)	104.3 %	95.7 %	97.6 %

TABLE 12 CBDA Precision, Accuracy, and Recovery

QC Sample	QC-LLOQ (ng/mL)	QC-1 (ng/mL)	QC-2 (ng/mL)
	10	30	1500
Batch 1			
Rep-1	10.41	27.8	1457
Rep-2	10.13	28.3	1472
Rep-3	10.68	28.0	1500
Rep-4	10.19	28.4	1491
Av.	10.353	28.13	1479.9
Precision (%)	2.4 %	1.1 %	1.3 %
Accuracy (%)	103.5 %	93.8 %	98.7 %
Batch 2			
Rep-1	10.60	27.0	1474
Rep-2	10.49	27.0	1446
Rep-3	10.56	27.7	1481
Rep-4	10.23	26.7	1458
Av.	10.469	27.10	1464.8
Precision (%)	1.6 %	1.5 %	1.1 %
Accuracy (%)	104.7 %	90.3 %	97.7 %
Batch 3			
Rep-1	10.24	28.6	1449
Rep-2	9.83	28.6	1481
Rep-3	10.03	28.6	1447
Rep-4	10.35	28.6	1478

QC Sample	QC-LLOQ (ng/mL)	QC-1 (ng/mL)	QC-2 (ng/mL)	QC Sample	QC-3 (ng/mL)	QC-LLOQ (ng/mL)	Cannabis CRM (mg/g)	QC-1 (ng/mL)	QC-2 (ng/mL)
	10	30	1500		8000	10	23.6	30	1500
Av.	10.114	28.62	1463.9	Rep-4	7880.8	10.46	24.0	28.6	1475
Precision (%)	2.3 %	0.1 %	1.2 %	Av.	0.6 %	10.249	4.4 %	28.98	1447.8
Accuracy (%)	101.1 %	95.4 %	97.6 %	Precision (%)	98.5 %	2.6 %	101.7 %	1.2 %	1.7 %
				Accuracy (%)		102.5 %		96.6 %	96.5 %

QC sample Inter-Batch Stats	QC-LLOQ	QC-1	QC-2	QC sample Inter-Batch Stats	QC-3	QC-LLOQ	Cannabis CRM	QC-1	QC-2
n	12	12	12	n	12	12	8	12	12
Av.	10.3	27.9	1470	Av.	7891	10.3	24.3	28.2	1467
Precision (%)	2.4 %	2.5 %	1.2 %	Precision (%)	1.0 %	2.1 %	3.5 %	2.7 %	1.7 %
Accuracy (%)	103.1 %	93.2 %	98.0 %	Accuracy (%)	98.6 %	102.9 %	103.1 %	94.0 %	97.8 %

TABLE 13 CBG Precision, Accuracy, and Recovery

QC Sample	QC-LLOQ (ng/mL)	QC-1 (ng/mL)	QC-2 (ng/mL)	QC Sample	QC-3 (ng/mL)	QC-LLOQ (ng/mL)	Cannabis CRM (mg/g)	QC-1 (ng/mL)	QC-2 (ng/mL)
	10	30	1500		8000	10	2.14	30	1500
Batch-1									
Rep-1	10.40	27.7	1456	Batch-1	8099		1.87		
Rep-2	10.51	28.8	1449	Rep-1	8021	10.22	2.08	28.0	1444
Rep-3	10.64	28.2	1462	Rep-2	8150	10.57	ALQ	27.9	1458
Rep-4	10.44	27.7	1483	Rep-3	8150	10.83	-	28.2	1453
Av.	10.496	28.11	1462.3	Rep-4	8105.1	10.57	1.98	28.4	1424
Precision (%)	1.0 %	1.9 %	1.0 %	Av.	0.8 %	10.547	7.3 %	28.13	1444.7
Accuracy (%)	105.0 %	93.7 %	97.5 %	Precision (%)	101.3 %	2.4 %	92.3 %	0.9 %	1.0 %
				Accuracy (%)		105.5 %		93.8 %	96.3 %
Batch-2									
Rep-1	10.54	28.0	1457	Batch-2	8096		2.02		
Rep-2	10.73	29.2	1461	Rep-1	8193	10.17	2.03	28.1	1434
Rep-3	10.83	28.3	1481	Rep-2	8048	10.78	2.00	28.6	1476
Rep-4	10.76	28.3	1488	Rep-3	8181	10.46	-	28.3	1476
Av.	10.718	28.43	1471.6	Rep-4	8129.6	10.51	2.02	27.8	1452
Precision (%)	1.2 %	1.8 %	1.0 %	Av.	0.9 %	10.480	0.8 %	28.18	1459.5
Accuracy (%)	107.2 %	94.8 %	98.1 %	Precision (%)	101.6 %	2.4 %	94.2 %	1.2 %	1.4 %
				Accuracy (%)		104.8 %		93.9 %	97.3 %
Batch-3									
Rep-1	10.40	28.6	1453	Batch-3	8218		2.06		
Rep-2	10.03	28.1	1422	Rep-1	8209	10.37	2.07	28.7	1443
Rep-3	10.43	27.5	1409	Rep-2	7923	10.15	2.02	29.7	1411
Rep-4	10.43	27.6	1446	Rep-3	8037	10.40	2.00	28.6	1445
Av.	10.321	27.97	1432.6	Rep-4	8096.7	10.14	2.04	29.2	1462
Precision (%)	1.9 %	1.8 %	1.4 %	Av.	1.8 %	10.262	1.5 %	29.06	1440.3
Accuracy (%)	103.2 %	93.2 %	95.5 %	Precision (%)	101.2 %	1.4 %	95.2 %	1.8 %	1.5 %
				Accuracy (%)		102.6 %		96.9 %	96.0 %

QC sample Inter-Batch Stats	QC-LLOQ	QC-1	QC-2	QC sample Inter-Batch Stats	QC-3	QC-LLOQ	Cannabis CRM	QC-1	QC-2
n	12	12	12	n	12	12	9	12	12
Av.	10.5	28.2	1455	n	8110	10.4	2.02	28.5	1448
Precision (%)	2.1 %	1.8 %	1.6 %	Av.	1.1 %	2.3 %	3.0 %	2.0 %	1.3 %
Accuracy (%)	105.1 %	93.9 %	97.0 %	Precision (%)	101.4 %	104.3 %	94.2 %	94.9 %	96.5 %
				Accuracy (%)					

TABLE 14 CBGA Precision, Accuracy, and Recovery

QC Sample	QC-LLOQ (ng/mL)	QC-1 (ng/mL)	QC-2 (ng/mL)	QC Sample	QC-3 (ng/mL)	QC-LLOQ (ng/mL)	Cannabis CRM (mg/g)	QC-1 (ng/mL)	QC-2 (ng/mL)
	10	30	1500		8000	10	4.25	30	1500
Batch-1									
Rep-1	10.06	28.1	1487	Batch-1	7827		3.99		
Rep-2	10.32	28.8	1483	Rep-1	7657	10.22	4.20	29.5	1461
Rep-3	10.69	28.1	1511	Rep-2	7943	10.09	ALQ	29.4	1502
Rep-4	10.27	27.7	1477	Rep-3	7654	10.92	-	30.6	1523
Av.	10.337	28.16	1489.3	Rep-4	7770.3	9.94	4.10	27.5	1326
Precision (%)	2.5 %	1.6 %	1.0 %	Av.	1.8 %	10.293	3.6 %	29.26	1453.3
Accuracy (%)	103.4 %	93.9 %	99.3 %	Precision (%)	97.1 %	4.2 %	96.4 %	4.4 %	6.1 %
				Accuracy (%)		102.9 %		97.5 %	96.9 %
Batch-2									
Rep-1	10.34	28.2	1454	Batch-2	7808		4.05		
Rep-2	10.32	27.2	1463	Rep-1	7742	10.33	4.13	27.9	1491
Rep-3	10.44	27.1	1455	Rep-2	7595	10.78	4.07	28.9	1446
Rep-4	10.03	27.4	1481	Rep-3	7595	10.50	-	26.7	1475
Av.	10.281	27.48	1463.2	Rep-4	7684.8	9.95	4.08	27.0	1353
Precision (%)	1.7 %	1.8 %	0.9 %	Av.	1.4 %	10.393	1.0 %	27.64	1441.4
Accuracy (%)	102.8 %	91.6 %	97.5 %	Precision (%)	96.1 %	3.4 %	96.1 %	3.6 %	4.3 %
				Accuracy (%)		103.9 %		92.1 %	96.1 %
Batch-3									
Rep-1	10.48	29.5	1463	Batch-3	7733		3.94		
Rep-2	10.12	28.9	1430	Rep-1	7585	10.29	4.04	28.6	1438
Rep-3	9.94	29.0	1424	Rep-2	7573	9.78	3.93	29.3	1392

TABLE 15 CBN Precision, Accuracy and Recovery

TABLE 16 CBNA Precision, Accuracy, and Recovery