INTERNATIONAL

Designation: 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.*

1.5 *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:*

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[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

[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.

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

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

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, 150 × 2.1 mm, 2.6 µm) with an analytical guard column (C18, 10 × 2.1 mm, 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, 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 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 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.

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	400	40.0
CBDA	1000	400	40.0
CBGA	1000	400	40.0
CBGVA	1000	400	40.0
CBNA	1000	400	40.0
THCVA	1000	400	40.0
CBCA	1000	400	40.0
CBDVA	1000	400	40.0
CBLA	500	800	40.0
Δ^9-THC	1000	400	40.0
CBD	1000	400	40.0
CBG	1000	400	40.0
CBGV	1000	400	40.0
CBN	1000	400	40.0
CBV	1000	400	40.0
THCV	1000	400	40.0
CBC	1000	400	40.0
CBDV	1000	400	40.0
CBL	1000	400	40.0
Δ^8-THC	1000	400	40.0
MeOH	-	1600	-
Total Volume	-	10 000	-

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	500	1500	2000	10 000
CAL-6	MCS-1	450	1550	2000	9000
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	150	3850	4000	1500

[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 4 ISWS Preparation

NOTE 1—Final volume may be changed provided the 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	-	48 250	-
Total volume	-	50 000	-

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 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, 2.6 µm, 2.1 mm × 150 mm HPLC column fitted with a 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.

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)	18
Column temperature (°C)	40
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

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 (Pa)	0.2
Dwell Time (msec)	40

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.

12.6 Centrifuge at 5000 r/min ± 500 r/min (4700 RCF ± 470 RCF) for 5 min ± 0.5 min.

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	2.6	**287**	**165**	**23**
		287	123	30
CBGVA	2.6	**315**	**191**	**23**
		333	191	26
CBV	3.5	**283**	**223**	**20**
		283	265	16
CBDA	3.7	**341**	**219**	**26**
		359	219	25
CBGA	4.0	**343**	**219**	**23**
		361	219	26
CBG	4.4	**317**	**193**	**16**
		317	123	32
THCV	4.4	**287**	**165**	**23**
		287	123	30
CBD	4.6	**315**	**193**	**21**
		315	135	20
THCVA	5.6	**313**	**191**	**26**
		313	233	20
CBN	6.5	**311**	**223**	**22**
		311	241	18
CBNA	7.8	**337**	**235**	**25**
		337	253	23
Δ9-THC	7.8	**315**	**193**	**21**
		315	135	20
Δ8-THC	8.3	**315**	**193**	**21**
		315	135	20
CBL	9.1	**315**	**235**	**18**
		315	81	30
THCA	9.6	**341**	**219**	**26**
		359	219	25
CBC	10.1	**315**	**193**	**21**
		315	259	14
CBLA	11.2	**359**	**261**	**25**
		359	219	32
CBCA	11.4	**341**	**219**	**26**
		359	219	25
CBGA-d3	4.0	346	222	23
CBG-d3	4.4	320	196	16
CBD-d3	4.5	318	196	21
CBN-d3	6.4	314	223	21
THC-d3	7.8	318	196	22
THCA-d3	9.6	344	222	26
CBCA-d3	11.4	362	222	25

12.7 Transfer the supernatant to clean 15 mL tubes.

12.8 Add a second 5 mL ± 0.1 mL aliquot of methanol:water (80:20, v:v) to the 15 mL tubes containing the cannabis matrix sample.

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

12.10 Centrifuge at 5000 r/min ± 500 r/min for 5 min ± 0.5 min.

12.11 Transfer the supernatant to the same 15 mL tube containing supernatant from the first extraction.

12.12 Vortex the supernatant for 5 s to 10 s.

12.13 *Dilutions:*

12.13.1 CAL and ICS solutions: No dilution required.

12.13.2 Blank, blank-0 and sample extracts: Dilute samples in methanol appropriately (Note 6).

12.14 Pipet 50 µL ± 1 µL of ISWS to glass screw cap vials with glass inserts (50 µL ± 1 µL of methanol to the blank).

12.15 Pipet 100 µL ± 2 µL of each sample to the vials.

12.16 Cap the vials and vortex for 10 s ± 5 s.

12.17 Analyze by LC-MS/MS.

NOTE 6—For example, a 1/500 dilution of extract provides quantitation in cannabis over a range of 0.5 mg/g to 500 mg/g and is achieved by pipetting 0.04 mL of supernatant and 19.96 mL of methanol to a glass vial and vortexing for 10 s ± 5 s.

13. Calculation or Interpretation of Results

13.1 Sample peak identities are verified by comparison of the retention time of analyte peaks in the appropriate SRM transitions of the ICS or a mid-level CAL with those of the samples. The peak area ratio (quantitation SRM transition/qualification SRM transition) of the samples shall be within ±30 % of the peak area ratio (quantitation SRM transition/qualification SRM transition) of the ICS or mid-level CAL.

13.2 The concentration of cannabinoids in the diluted samples (ng/mL) are calculated from the weighted calibration curve of the peak area ratios (analyte/internal standard). If the cannabinoid concentration in the diluted sample is determined to be below the calibration range, it is reported as below the limit of quantitation (BLQ) or the sample extract may be diluted at a lower level to obtain a concentration within the calibration curve range and re-analyzed. If the cannabinoid concentration in the diluted sample is determined to be above the calibration range, the sample extract shall be re-diluted at an appropriate level to obtain a concentration near the midpoint of the calibration range and re-analyzed. All samples with concentrations within the calibration range are reported. The concentrations (mg/g) of cannabinoids in cannabis are then determined by accounting for sample mass, extraction, dilution and water content (see Test Method E203) as shown in Eq 2.

$$Cm = (Cd)(df)(Ve) / Mm(1000000)(1 - W) \quad (2)$$

where:

Cm = concentration of cannabinoid in the cannabis sample in mg/g,
Cd = concentration of cannabinoid in the diluted sample in ng/mL,
df = dilution factor,
Ve = extract volume in mL,
Mm = mass of the cannabis sample used in g,
1 000 000 = number of ng in one mg, and
W = water content in the cannabis sample in g/g.

Cannabinoid content in cannabis without water content correction may be calculated by setting W=0.

13.3 Total cannabinoid concentration (mg/g) in cannabis, for each neutral cannabinoid–acidic cannabinoid pair, is calculated as the total neutral cannabinoid equivalent using the neutral cannabinoid concentration in mg/g, the acidic cannabinoid concentration in mg/g, the neutral cannabinoid molecular weight and the acidic cannabinoid molecular weight as shown in Eq 3.

$$Cmt = Cmn + Cma\left(\frac{MWn}{MWa}\right) \quad (3)$$

where:

Cmt = concentration of total cannabinoid (neutral cannabinoid equivalent) in the cannabis sample in mg/g,
Cmn = concentration of neutral cannabinoid in the cannabis sample in mg/g,
Cma = concentration of acidic cannabinoid in the cannabis sample in mg/g,
MWn = molecular weight of the neutral cannabinoid, and
MWa = molecular weight of the acidic cannabinoid.

13.4 Analyte concentrations are recorded in the appropriate documentation as defined by your QMS.

14. Precision and Bias

14.1 The determination of precision and accuracy is based on data from a single-laboratory validation[3] (see Practice D8282). A multi-laboratory validation will be planned and it is hoped that this test method will generate multi-laboratory participants to enable a full validation study within the next 5 years.

14.2 Method validation data is discussed in more detail in the publication[3] and includes evaluation of specificity/LLOQ, selectivity, recovery, ion suppression, linearity, quality control (QC) sample precision and accuracy (bias), cannabis CRM precision and accuracy (bias), solution stability, extract stability, processed sample stability as well as repeatability and intermediate precision of seven (7) cannabis samples.

14.3 *Single Laboratory Validation*—Intra-batch and inter-batch precision, accuracy, and recovery were demonstrated for twenty (20) cannabinoids in quality control (QC) samples in methanol in three independent batches, precision and recovery were demonstrated for sixteen (16) cannabinoids in cannabis matrix CRMs in three independent batches, and routine recovery (RRS) was demonstrated for eighteen (18) cannabinoids in cannabis matrix. (Note 7) (Tables 9-28).

NOTE 7—Accuracy results for the matrix CRM samples represent % recovery of cannabinoids from matrix calculated as percentage of the determined analytical concentration relative to the certificate of analysis concentration. Results are reported for those cannabinoids that have concentration values on the certificate of analysis. RRS recovery was performed as described in 10.2.6, and results are reported for those cannabinoids that exhibited appropriate concentrations in the matrices.

14.4 Triplicate extractions of seven cannabis samples were analyzed in a single batch to evaluate repeatability. This process was repeated on three separate days to evaluate intermediate precision. The seven cannabis samples represented high THC-THCA, high CBD-CBDA and balanced THC-THCA, CBD-DBDA matrix concentrations, (Table 29).

14.5 Additional validation work was completed to evaluate a larger sample extract dilution to extend the upper matrix concentration to 500 mg/g. Triplicate preparations of sample extract dilutions of 1/500 and 1/200 were evaluated. Precision results for Δ9-THC, Δ9-THCA, CBD, CBDA, CBG, CBGA, CBN, CBNA, CBC, and CBCA were 0.4 % to 6.3 % for the 1/200 dilution and 0.2 % to 2.8 % for the 1/500 dilution. Concentration results for the 1/500 and 1/200 dilutions were within ±3.2 % of each other.

15. Keywords

15.1 analyses; cannabinoids; cannabis; hemp; laboratory; LC-MS/MS

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)	Hemp CRM (mg/g)	Cannabis CRM (mg/g)	HEMP RRS Recovery	Cannabis RRS Recovery
	10	30	1500	8000	0.318	48.9	(%)	(%)
Batch-1								
n	6	6	6	6	6	6	-	-
Av.	8.41	27.8	1474	7428	0.279	50.3	-	-
Precision (%)	0.7 %	1.5 %	0.8 %	0.2 %	0.5 %	0.9 %	-	-
Accuracy (%)	84.1 %	92.5 %	98.3 %	92.8 %	87.8 %	103.0 %	-	-
Batch-2								
n	6	6	6	6	6	6	-	-
Av.	9.17	29.2	1529	7644	0.278	46.0	-	-
Precision (%)	1.0 %	1.3 %	0.6 %	0.8 %	0.8 %	%	-	-
Accuracy (%)	91.7 %	97.5 %	101.9 %	95.6 %	87.3 %	94.0 %	-	-
Batch-3								
n	6	6	6	6	6	6	1	-
Av.	9.00	27.6	1503	7368	0.275	46.3	-	-
Precision (%)	1.1 %	0.6 %	0.5 %	0.3 %	0.9 %	0.7 %	-	-
Accuracy (%)	90.0 %	92.1 %	100.2 %	92.1 %	86.4 %	94.8 %	95.6 %	-
QC sample Inter-Batch Stats								
n	18	18	18	18	18	18	-	-