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:*

[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