Analysis of Delta-9-tetrahydrocannabinol

1 Scope

This procedure is used for the analysis of items suspected of containing delta-9-tetrahydrocannabinol (Δ^9-THC or THC, the major psychoactive compound in marijuana). This procedure may also be used to identify a vegetative substance as cannabis, however the procedure does not allow for the identification of the substance as marijuana or hemp.

This procedure applies to Chemistry Unit (CU) personnel that are qualified and authorized to examine General Chemistry evidence for the presence of drugs.

2 Equipment/Materials/Reagents

- Common laboratory glassware and equipment
- Analytical balance
- Evaporator
- Stereoscope
- Digital Microscope (Keyence or equivalent)
- Silica gel Thin-Layer Chromatography (TLC) plate and TLC tank
- Petroleum Ether
- Methanol (MeOH)
- Toluene
- Chloroform
- Deionized water
- Fast Blue BB Salt
- Polyethylene glycol (PEG, 550 average molecular weight)
- Delta-9-tetrahydrocannabinol (Δ^9-THC)
- Cannabinol (CBL)
- Cannabidiol (CBD)
- Time-of-flight mass spectrometer with direct analysis in real time ionization source (DART/TOFMS)
- Gas chromatograph/mass spectrometer (GC/MS) equipped with electron impact ionization and a 30 meter DB-5 column (or equivalent)

3 Preparation of Color Test Reagent

3.1 Fast Blue BB Solution

Dissolve 15 mg of Fast Blue BB salt into 20 mL of deionized water. Prepare fresh with each use. Handle with caution, Fast Blue BB salt is a known carcinogen.

4 Standards and Controls

4.1 Negative Control

The same volume of solvent from the same source and lot used to extract the questioned item(s) and within a similar container (e.g., test tube, vial) will be used as the Negative Control.

4.2 Positive Controls

- Δ^9-THC Solution (100 ug/mL):

  Prepared by diluting a 1 mg/mL certified reference material 1:10 in an appropriate solvent. This solution will be stored in a freezer and is verified with each use. This solution may be further diluted (e.g., 50 ug/mL) to prevent overloading an instrument.

- Other Cannabinoids:

  Cannabinol (CBL) and cannabidiol (CBD) can also be used as Positive Controls as needed. Prepared as 100 ug/mL solutions by dissolving a 1 mg/mL certified reference material 1:10 in an appropriate solvent. Other concentrations may be prepared as needed. Solutions will be stored in a freezer and are verified with each use.

5 Sampling

Statistical sampling is performed according to the General Chemistry *Sampling Guidelines for Bulk Materials and Multi-Unit Populations* (GenChem 21).

When non-statistical sampling is utilized on a heterogeneous item, the results of examinations will be clearly limited to the sample(s) that was selected and examined.

6 Procedure

Refer to *General Chemistry Instrument Parameters* (GenChem 34) for specific instrument settings and decision criteria.

a. Use a traceable analytical balance to record the weight for each item, as applicable.

b. Perform a visual examination of each item. Examples of residue/ paraphernalia items include burnt substances within pipes or cigarettes, finely ground vegetative material within 'grinders', and resinous semi-solids. Food products may also be analyzed for Δ^9-THC.

c. The odor, or any other significant characteristics of the item, should be noted. Cannabis is typically green, brown, or a variation between the two.

d. Perform a microscopic examination of any potentially intact vegetative substance for the presence of cystolithic and glandular hairs. The shorter, "bear claw" shaped hairs are the cystolithic hairs, while the glandular hairs (also referred to as trichomes) often contain beads of resin on the end.

e. Weigh (record the weight) and transfer ~ 10 mg of the item to a labeled test tube. An item may have packaging with Δ^9-THC concentration information; this information may be used to determine an alternative amount to weigh and transfer. Extract with ~ 1 mL of an appropriate solvent (e.g., petroleum ether, MeOH). Vortex the solution and allow the item to extract for ~ 5 minutes. Use an empty, labeled test tube as a Negative Control.

f. Transfer the extracts to new labeled test tubes. If necessary, concentrate the extracts under N_2 (g) flow at 60 ºC.

g. Analyze the extracts by DART/TOFMS in the positive ionization mode by sampling the extracts with the closed end of a glass capillary. Analyze the Negative Control(s), the Δ^9-THC Positive Control, and PEG within the same data collection file.

h. As an alternative to DART/TOFMS, the extracts may be analyzed by TLC. Fill a TLC tank with an appropriate amount of mobile phase [100% toluene; petroleum ether:chloroform (60:40 volume:volume); or toluene:chloroform (50:50 volume:volume)] and allow it to equilibrate. Spot 5 to 10 uL of the extract(s) at the origin of a silica gel TLC plate (typically ≥ 1 cm from bottom of TLC plate). Spot 5 to 10 uL of the Δ^9-THC Positive Control and the extracts on the same TLC plate. Allow the spots to dry. Place the TLC plate into the TLC tank and allow the mobile phase to migrate ~ 10 cm up the plate. Remove the TLC plate from the tank, mark the location of the mobile phase solvent front and allow the plate to dry.

Develop the plate by applying the Fast Blue BB solution via an aerosol sprayer. Red spots indicate the possible presence of cannabinoids. Record and/or photograph the results. Calculate the retardation factor (R_f) for any red spots that are observed.

i. Analyze the extracts and Δ^9-THC Positive Control by GC/MS using electron impact (EI) ionization mode. Incorporate a solvent blank between each sample.

7 Calculations

- R_f = (distance spot center traveled from origin) / (distance of solvent front from origin)
- Distances traveled are measured from the origin. Distances are considered approximate and the R_f value is not treated as a significant measurement.

8 Measurement Uncertainty

When quantitative results (e.g., weight, volume) are included in a *Laboratory Report*, measurement uncertainty will be estimated and reported following the *Chemistry Unit Procedures for Estimating Measurement Uncertainty* (CU QAOM 13). Uncertainty budget worksheets for each analytical balance approved for significant measurements are maintained electronically in CU.

9 Limitations

- The available sample size may limit or preclude some analytical techniques from being performed.
- Older items may have minimal amounts of Δ^9-THC, which may cause TLC to give weak or negative results and/or may require an increase in the amount of substance to sample and extract.
- Testing of an item for identification of cannabis as hemp or marijuana (or as derived from hemp or marijuana) is not conducted in the CU.

The following conclusions apply to the analysis of items for delta-9-tetrahydrocannabinol:

- Identification (i.e. identified)
- Consistent with
- Not identified
- Inconclusive

Refer to *Chemistry Unit (CU) FBI Approved Standards for Scientific Testimony and Report Language for General Chemistry* (GenChem 32, ASSTR), *General Approach to Report Writing in General Chemistry* (GenChem 27), and *Department of Justice Uniform Language for Testimony and Reports for General Forensic Chemistry and Seized Drug Examinations* (GenChem ULTR) for examples of reporting examination conclusions and the associated limitations and decision criteria.

Refer to *General Chemistry Instrument Parameters* (GenChem 34) for instrumental limitations and decision criteria.

Refer to *General Chemistry Guidelines for Comparison of Mass Spectra* (GenChem 33) for mass spectra comparison decision criteria.

10 Safety

- Take standard precautions for the handling of all chemicals, reagents, and standards. Some of the chemicals may be carcinogenic. Refer to the *FBI Laboratory Safety Manual* for the proper handling and disposal of all chemicals. Personal protective equipment should be used when handling any chemical and when performing any type of analysis.
- Fast Blue BB salt is a known carcinogen and should be handled with care.

11 References

Agriculture Improvement Act of 2018. Public Law No: 115-334 (12/20/2018). 115th Congress Public Law 334.

Implementation of the Agriculture Improvement Act of 2018 (DEA 'Interim final rule'). Department of Justice, Drug Enforcement Administration. 21 CFR Parts 1308 and 1312, [Docket No. DEA-500], RIN 1117-AB53.

Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), *SWGDRUG Recommendations*, 8th Edition, 2019

Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), *SWGDRUG Monographs*, www.swgdrug.org

Moffat, AC. *Clarke's Isolation and Identification of Drugs*, 3rd ed., Pharmaceutical Press: London, 2004

European Network of Forensic Science Institutes (ENFSI), *Guidelines on Representative Drug Sampling*, 2009

European Network of Forensic Science Institutes (ENFSI), *Guidelines on Sampling of Illicit Drugs for Qualitative Analysis*, 2nd Edition, 2016

Agriculture Improvement Act of 2018 (12/20/18). Subtitle G – Hemp Production, Sec. 297A. Definitions. "(1) HEMP.-The term 'hemp' means the plant Cannabis sativa L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis."

Sampling Guidelines for Bulk Materials and Multi-Unit Populations; FBI Laboratory Chemistry Unit – General Chemistry SOP (GenChem 21)

General Chemistry Instrument Parameters; FBI Laboratory Chemistry Unit – General Chemistry SOP (GenChem 34)

Guidelines for Comparison of Mass Spectra; FBI Laboratory Chemistry Unit – General Chemistry SOP (GenChem 33)

Chemistry Unit Procedures for Estimating Measurement Uncertainty; FBI Laboratory Chemistry Unit – Quality Assurance and Operations Manual (CU QAOM 13)

FBI Laboratory Safety Manual

Rev. #	Issue Date	History
4	01/15/20	Revised title. Removed content concerning the identification of vegetative substance as marijuana. Removed previous section 1 (Introduction), section 3 (Principle), and section 6 (Calibration), and renumbered accordingly. Edited new section 1 for clarity and to include personnel. Defined 'Chemistry Unit' as 'CU'. Changed lettered listing in section 2 to bullets and revised the list. Removed Duquenois-Levine color test from new section 3 and made minor edits. Minor content and format changes made to new section 4. Removed 'subunit' from new section 5 reference. Revised new section 6 (primarily stylistic): DART/TOFMS is the primary screening technique, TLC becomes backup screen, and removed Duquenois-Levine color test. Revised new section 7.1 for clarity. Revised section 9 for clarity and added flexibility. Minor edits to Measurement Uncertainty section, removed location of budget worksheet, moved to earlier in the document. Removed approximate LODs from section 12. Updated references section (content and format).
5	04/01/21	Section 1- added second sentence addressing cannabis; edited last sentence to include only General Chemistry evidence; and added "and authorized". Section 5- minor edit to first sentence; second sentence- added "on a heterogeneous item". Section 6- added first sentence; added steps (c) and (d); steps (f), (h), and (i)- edited for clarity. Added detail to section 7 and clarified that the distance measurements are approximate and thus measurement uncertainty is not applicable for R_f values. Deleted previous sections 7.1, 9, and 10. Section 8- edited for clarity and to remove "s:\ drive". Section 9- added the third bullet in the first list and the content below the first bulleted list. Section 11- added first 2 references; added GenChem 34; added parenthetical references to end of CU SOPs.

Redacted - Signatures on File

Approval

Chemistry Unit Chief: Date: 03/31/2021

General Chemistry
Technical Leader: Date: 03/31/2021