

Synthetic Cannabinoid Analysis

1 Scope

This procedure allows for the analysis of items suspected of containing synthetic cannabinoids. Typical items for analysis include herbal blends, solids, liquids, and residual materials from smoking devices. Direct analysis in real time/time-of-flight mass spectrometry (DART/TOFMS), gas chromatography/mass spectrometry (GC/MS), and/or liquid chromatography/mass spectrometry (LC/MS) results are compared to acquired reference materials (when available) that are analyzed on the same equipment under the same conditions.

This procedure applies to General Chemistry personnel in the Chemistry Unit that are qualified to examine evidence for the presence of synthetic cannabinoids.

2 Equipment/Materials/Reagents

- Common laboratory glassware and equipment
- Analytical balance
- Stereo microscope
- Digital microscope
- Time-of-flight mass spectrometer with direct analysis in real time ionization source (DART/TOFMS)
- Gas chromatograph/mass spectrometer (GC/MS) equipped with an electron impact ionization source and a 30 meter DB-5 column (or equivalent)
- Liquid chromatography system with Waters Xterra C18 MS column (or equivalent) coupled to a mass spectrometer (LC/MS) with electrospray ionization (ESI) (e.g., Thermo LTQ, Thermo LTQ OrbiTrap XL, Thermo Exactive OrbiTrap)
- Acetonitrile
- Deionized water
- Ethanol
- Formic acid
- Methanol
- Polyethylene glycol (PEG, 550 average molecular weight)
- Synthetic cannabinoid reference materials

3 Standards and Controls

3.1 Negative Control

A Negative Control will be prepared by mirroring the process used to prepare a sample from a questioned item. For example, use the same volume of methanol from the same source and lot and within a similar container used to extract the questioned item(s). It is left to the discretion of the examiner as to what constitutes an adequate Negative Control.

3.2 Positive Controls

All Positive Controls will be verified at the time of use. The amounts of materials indicated in this section may be scaled up or down as necessary.

3.2.1 Synthetic Cannabinoid Positive Control (100 ug/mL)

A synthetic cannabinoid Positive Control is prepared at 100 ug/mL for analysis by DART/TOFMS and GC/MS by dissolving the stock reference material solution in methanol. Stock reference material solutions are typically purchased at concentrations of 1.0 or 5.0 mg/mL. For example, dilution of 20 uL of a 5.0 mg/mL stock solution of JWH-073 to a final volume of 1 mL will yield a 100 ug/mL solution of JWH-073.

Store the stock solutions and Positive Controls in glass containers in a freezer.

3.2.2 Synthetic Cannabinoid Positive Control (1 ug/mL)

A synthetic cannabinoid Positive Control is prepared at 1 ug/mL for analysis by LC/MS by dissolving a 100 ug/mL Positive Control or stock reference material solution in methanol. For example, dilution of 10 uL of a 100 ug/mL solution of JWH-073 to a final volume of 1 mL will yield a 1 ug/mL solution of JWH-073.

Store the stock solutions and Positive Controls in glass containers in a freezer.

4 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 were selected and examined.

5 Procedure

- a. Use a traceable analytical balance to record the weight for each item, as applicable.
- b. Perform a visual and microscopic examination of each item, where applicable. For a vegetative item, particularly residue from within a smoking device, the potential exists for cannabis (marijuana) to be mixed with the item. If vegetative substance consistent with cannabis is observed, refer to *Analysis of Delta-9-tetrahydrocannabinol* (GenChem 8) in addition to this procedure.
- c. For a vegetative item, weigh out approximately 100 mg by combining multiple samples from the item and transfer to a labeled test tube. If the item does not contain enough substance to create a 100 mg sample, make note of the weight of the item that was sampled.
- d. For an unknown solid material, weigh out approximately 10 mg of the substance and transfer to a labeled test tube.
- e. For an unknown liquid preparation, thoroughly mix the solution and transfer 20 uL to a labeled test tube. Alternatively, the liquid may be analyzed neat by DART/TOFMS [see step (k) below] and then diluted based on the observed signal intensity.
- f. For a paraphernalia item (e.g., smoking device) that is mostly empty, rinse the item with 1 mL of methanol or acetonitrile and transfer the rinse to a labeled test tube. Use an empty, labeled test tube as a Negative Control and add the same volume of solvent from the same lot that was used to rinse the questioned item. Proceed to step (i).
- g. Use an empty, labeled test tube as a Negative Control.
- h. Add 1 mL of methanol or acetonitrile to the Negative Control and questioned item(s) test tubes [from steps (c), (d), and/or (e) above]. Vortex mix each sample, then allow the samples to extract for ~ 5 minutes.
- i. For a rinse of a paraphernalia item, or for items with limited sample amounts, concentrate the applicable Negative Control(s) and questioned item(s) under N₂ (g) flow at ~60 °C to a final volume of ~100 uL. If a sample was taken to dryness, reconstitute with 100 uL of methanol or acetonitrile.

- j. If necessary, filter the Negative Control and questioned item(s) with 0.2 um PTFE syringe filters that have been pre-rinsed with methanol or acetonitrile. Collect the filtrates in new, labeled test tubes.
- k. Analyze the Negative Control(s) and questioned item(s) by DART/TOFMS in positive ionization mode. The closed end of a glass capillary is typically used to introduce the sample to the DART source. PEG will be analyzed with each data collection file to allow for mass-to-charge correction. Acquire and prepare the applicable 100 ug/mL synthetic cannabinoid(s) Positive Control(s) and analyze by DART/TOFMS in positive ionization mode. This can be done after step(s) (l) and/or (m) if necessary.
- l. Analyze the Negative Control(s), questioned item(s), and the applicable 100 ug/mL synthetic cannabinoid(s) Positive Control(s) [if the suspected synthetic cannabinoid(s) are known at this point in the analysis] by GC/MS. Incorporate methanol or acetonitrile blanks between each sample. If the data from DART/TOFMS indicated a heavily concentrated questioned item extract, appropriately dilute the extract(s) in methanol or acetonitrile to achieve a synthetic cannabinoid concentration of ~100 ug/mL. If the suspected synthetic cannabinoid(s) were unknown prior to analyzing by GC/MS, or if additional synthetic cannabinoids are suspected based on the GC/MS analysis, acquire and prepare the applicable 100 ug/mL synthetic cannabinoid(s) Positive Control(s) and analyze by GC/MS. Incorporate methanol or acetonitrile blanks between each sample.
- m. If necessary, the Negative Control(s), questioned item(s), and the applicable 1 ug/mL synthetic cannabinoid(s) Positive Control(s) may be analyzed by LC/MS (ESI). Incorporate methanol or acetonitrile blanks between each sample. Questioned item(s) should be diluted to achieve a synthetic cannabinoid concentration of ~5 ug/mL.

6 Calculations

Not applicable

7 Measurement Uncertainty

Not applicable

8 Instrumental Conditions

Refer to *General Chemistry Instrument Parameters* (GenChem 34) for specific instrument settings and decision criteria that are not provided below.

The following instrumental conditions are not intended to be prescriptive nor exhaustive. Minor modifications to the conditions may be used as needed and without authorization, provided the same conditions are used for all applicable solvent blanks, control samples, and questioned items; and the Positive Control(s) provide acceptable data. The utilized conditions will be recorded and retained with the case notes.

8.1 Liquid Chromatography/Mass Spectrometry (LC/MS)

8.1.1 Liquid Chromatography Parameters

Mobile Phase Compositions		Flow Parameters			Column Parameters	
A: 0.1% formic acid in acetonitrile		total flow = 0.25 mL/min			type	Waters Xterra C18 MS
		time (min)	% A	% B	length	100 mm
B: 0.1% formic acid in water		0	30	70	internal diameter	3.0 mm
		3.0	30	70	particle size	3.5 μ m
		15.0	90	10	temperature	30 °C
Autosampler		30.0	90	10		
temperature	15 °C	31.0	30	70		
injection volume	5 μ L	36.0	30	70		
		total run time = 37 min.				

8.1.2 Mass Spectrometer Parameters

8.1.2.1 High Resolution Full Scan (Resolution = 30,000)

Ionization Mode	ESI (+)
Scan Mode	FTMS res=30000
Scan Range	250-650 m/z
Source parameters are set through the tune file and should be optimized on each instrument. Retain a copy of the tune parameters with the case notes.	

8.1.2.2 Tandem Mass Spectrometry (MS/MS)

Scan Event #1		Scan Event #2	
Ionization Mode	ESI (+)	Ionization Mode	ESI (+)
Scan Mode	FTMS res=7500	Scan Mode	FTMS res=7500; MS/MS
Scan Range	250-650 <i>m/z</i>	Precursor	Target analyte dependent
Source parameters are set through the tune file and should be optimized on each instrument. Retain a copy of the tune parameters with the case notes.		Isolation Width	2.0
		Collision Energy	35%
		Activation Q	0.250
		Activation Time	30.0
		Scan Range	software control

9 Limitations

The following conclusions apply to the analysis of synthetic cannabinoids:

- 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.

11 References

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Analysis of Delta-9-tetrahydrocannabinol; FBI Laboratory Chemistry Unit – General Chemistry SOP (GenChem 8)

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)

Chemistry Unit (CU) FBI Approved Standards for Scientific Testimony and Report Language for General Chemistry – General Chemistry SOP (GenChem 32)

General Approach to Report Writing in General Chemistry; FBI Laboratory Chemistry Unit – General Chemistry SOP (GenChem 27)

Department of Justice Uniform Language for Testimony and Reports for General Forensic Chemistry and Seized Drug Examinations (GenChem ULTR)

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

FBI Laboratory Safety Manual

Rev. #	Issue Date	History
0	12/05/11	Original document.
1	08/17/20	Removed previous sections 1 (Introduction), 3 (Principle), 4 (Specimens), 7 (Calibration), and 11 (Decision Criteria, including Table 1); renumbered sections accordingly. Edited new section 1 for clarity and to include personnel. Scope expanded to the broad class of synthetic cannabinoids, previous revision was specific to seven compounds. Comparison to reference materials added to scope. Changed lettered listing in new section 2 to bullets and revised the list. Edited new section 3 to include broader range of synthetic cannabinoids; changed formatting. Added content to section 4 (Sampling) and updated reference. Edited content of section 5 for clarity and to include broader range of synthetic cannabinoids. Changed new section 7 title from 'Uncertainty of Measurement'. Section 8 edited to refer to GenChem 34 for instruments except LC/MS. Minor formatting changes to LC/MS parameters. Section 9 edited to include conclusion statements and references to ASSTR, ULTR, etc. Updated references in section 11.

Approval

Redacted - Signatures on File

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