

## Analysis of Bank Security Device Chemicals

### 1 Scope

Redacted

This procedure applies to Chemistry Unit (CU) personnel that are qualified to examine evidence for the presence of bank security device chemicals.

### 2 Equipment/Materials/Reagents

- Common laboratory glassware and equipment
- Analytical balance
- Acetone
- Redacted
- Redacted
- Chloroform
- Deionized water
- Redacted
- Methanol (MeOH)
- 1-Methylaminoanthraquinone (MAAQ)
- Orthochlorobenzalmalononitrile (CS tear gas)
- Toluene
- Evaporator
- Silica gel Thin-Layer Chromatography (TLC) plate and TLC tank
- Polyethylene glycol (PEG, 550 average molecular weight)
- 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)
- Gas chromatograph/mass spectrometer (GC/MS) equipped with chemical ionization and a 30 meter DB-5 column (or equivalent)

### 3 Standards and Controls

#### 3.1 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. If swabs are submitted as evidence, a blank swab extracted in the same manner as the questioned item(s) will be used as the Negative Control. In some instances an extract from a portion of the items(s) which does not contain a stain of interest may be selected for use as a Negative Control.

#### 3.2 Positive Controls

- MAAQ Stock Solution (1 mg/mL):

Prepared by dissolving 10 mg of MAAQ in 10 mL of an appropriate solvent (e.g. acetone, MeOH). This solution will be stored in a freezer.

- MAAQ/CS Positive Control Solution (50 ug/mL):

Prepared by weighing out 5 mg of orthochlorobenzalmalononitrile (\*Lachrymator-use caution when handling), adding 5 mL of the above MAAQ Stock Solution (1 mg/mL), and diluting with an appropriate solvent to a final volume of 100 mL. This solution will be stored in a freezer and is verified with each use. This solution may be further diluted (e.g., 5 ug/mL) to prevent overloading an instrument.

- Redacted

## 4 Sampling

Typically, one or more samples (e.g., cuttings, swabbings) are selected from the stained area(s) of the questioned item. When multiple samples are selected from the same item, the samples are typically combined prior to extraction.

Multiple items that are packaged together or otherwise in contact with each other will typically be sampled as one collective item. For example, if ten bills of currency are packaged together, one or more of the bills will be sampled and the selected samples will be combined prior to extraction.

When non-statistical sampling is utilized, the results of examinations will be clearly limited to the sample(s) that were selected and analyzed.

## 5 Procedure

- a. Thoroughly examine items for red or pink stains, Redacted
- b. If a red or pink stain is observed, cut a small section of the item which contains the stain of interest and transfer the cutting to a labeled test tube. Multiple cuttings from the same item may be combined. Use an empty, labeled test tube as a Negative Control.
- c. If the stain is not conducive to cutting, remove a sample of the stain by rubbing a cotton swab that has been wetted with a few drops of an appropriate solvent (e.g., acetone, MeOH) over the stain. If the stain is very dark and has a powdery appearance, it can be sampled with a dry cotton swab (or scraped directly into a test tube). Transfer the swab to a labeled test tube. Additionally, transfer an unused swab from the same source (dry or wetted with the same solvent) to a labeled test tube as a Negative Control.
- d. If the item is dark in color such that a red and/or pink stain could be obscured, rub cotton swabs (dry or wetted with an appropriate solvent) over the item. Examine the swab for any red or pink color that may have transferred to the swab. Swab as necessary until either the entire item has been swabbed or until a red or pink color has transferred to the swab. Transfer the swab(s) to a labeled test tube. Additionally, transfer an unused swab from the same source (dry or wetted with the same solvent) to a labeled test tube as a Negative Control.
- e. If no red or pink stains are observed on an item, or if no stain was transferred to a swab for a dark colored item (step d), then additional exams may be omitted.

- f. Test the solubility of the stain using an appropriate amount of solvent (e.g., acetone, MeOH) added to the test tube containing the selected sample(s). Do not use acetone if the item is plastic. Spot check a small portion of the plastic substrate with chloroform. If the plastic does not dissolve in chloroform, then chloroform may be substituted for acetone. If the chloroform dissolves the plastic, use MeOH. The Negative Control sample(s) shall be extracted in the same manner as the selected sample(s).
- g. Isolate the extract solutions by transferring the solutions to new labeled test tubes. Note the color of the extracts, to include the Negative Control(s).
- h. If the extract solution(s) is colorless or faint/light pink, concentrate the extract(s) under N<sub>2</sub> (g) flow at ~60 °C. The extract(s) should not be taken to dryness due to the possible loss of CS tear gas. Concentrate the associated Negative Control extract(s) in the same manner as the extract(s) from the questioned item(s). If the extract solution(s) is colorless after concentration then the analysis is complete.
- i. Analyze 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 MAAQ/CS Positive Control, and PEG within the same data collection file. Redacted

If a questioned item extract does not indicate the presence of MAAQ R  
by DART/TOFMS then additional exams may be omitted. e  
d

- j. As an alternative to DART/TOFMS, the extracts may be analyzed by TLC. Fill a TLC tank with an appropriate amount of toluene 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 the MAAQ/CS Positive Control and the Negative Control(s) extract(s) on the same TLC plate. Redacted

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. Record and/or photograph the results. Calculate the retardation factor (R<sub>f</sub>) for any pink/red spots that are observed. If a questioned item extract does not indicate the presence of MAAQ Redacted then additional exams may be omitted.

- k. Analyze the extracts by GC/MS using electron impact (EI) ionization mode. Also analyze the Negative Control(s) and the MAAQ/CS Positive Control, and incorporate a solvent blank between each sample. Redacted

Redacted

1. Analyze the extracts by GC/MS using negative ion chemical ionization (NICI) mode. This will be conducted even if the above analysis by GC/MS (EI) was negative for CS tear gas since NICI is the more sensitive technique. Also analyze the Negative Control(s) and the MAAQ/CS Positive Control, and incorporate a solvent blank between each sample. Redacted

## 6 Calculations

### 6.1 Thin-layer Chromatography (TLC)

- Distances traveled are measured from the origin
- *Retardation factor* ( $R_f$ ) =  $\frac{\text{distance traveled by center of spot}}{\text{distance traveled by solvent}}$

## 7 Measurement Uncertainty

Not applicable

## 8 Instrumental Conditions

The following instrumental conditions are typically used for this analysis. Minor modifications to the conditions may be used as needed and without authorization, provided the same conditions are used for all 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. [Note- some of the parameters are unique to an individual instrument and will be different among similar instruments (e.g., column specifics, tune parameters).]

## 8.1 DART/TOFMS

DART Source Settings	
Polarity	Positive
Source gas	Helium
Temperature	400 °C

Redacted

TOFMS Settings	
Polarity	Positive
Ion Guide RF (or equivalent)	800 V for 80-800 $m/z$ , 500 V for 50-500 $m/z$

Redacted

## 8.2 Gas Chromatography/Mass Spectrometry (GC/MS)

### 8.2.1 Electron Impact (EI)

GC Settings	
Injection Mode	Split or Splitless (sample dependent; consistent across blanks, controls, samples)
Injection Volume	1 uL
Inlet Temperature	250 °C
Oven Program	60 °C for 2 min, 35 °C /min to 260 °C for 15 min
MS Settings	
Polarity	Positive
Scan Range	43 to 400 $m/z$

### 8.2.2 Chemical Ionization (CI)

GC Settings	
Injection Mode	Split or Splitless (typically Splitless, but may be sample dependent; consistent across blanks, controls, samples)
Injection Volume	1 uL
Inlet Temperature	250 °C
Oven Program	60 °C for 2 min, 35 °C /min to 260 °C for 15 min
MS Settings	
Polarity	Negative
Reagent Gas	Methane
Scan Range	100 to 400 $m/z$

## 9 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of data produced in this procedure.

### 9.1 Thin Layer Chromatography

The  $R_f$  value(s) of the spot(s) from the questioned item(s) should be within  $\pm 10\%$  of the  $R_f$  value(s) of the spot(s) for the corresponding Positive Control(s).

### 9.2 DART/TOFMS

Peaks of interest should be within  $\pm 0.005$   $m/z$  of a contemporaneously analyzed Positive Control and/or the theoretical accurate mass value of the ion of interest.

### 9.3 Gas Chromatography/Mass Spectrometry

- Peaks should show good chromatographic characteristics with reasonable peak shape, width, and resolution.
- The retention time of a compound of interest should be within  $\pm 2\%$  of the retention time of a contemporaneously analyzed Positive Control.
- The signal intensity (integrated area) for a peak of interest should be  $\geq 10$  times the signal intensity for a peak of the same compound present in an injection immediately prior, as well as in the associated Negative Control(s).
- The mass spectrum of the peak of interest should compare favorably with that of a contemporaneously analyzed Positive Control. See *Guidelines for Comparison of Mass Spectra* (GenChem 33) for further guidance.

## 10 Limitations

- CS tear gas is present in a smaller concentration in bank security devices than MAAQ and is a volatile chemical.
- In instances where items are negative for MAAQ or where visible pink/red color stains are absent, no further examinations are performed for bank security device chemicals.
- MAAQ strongly binds to polymeric materials (e.g., styrene, methylmethacrylate). This may limit solvent choices and the ability to extract MAAQ.

## 11 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.
- CS tear gas is a lachrymator and should be handled carefully.

## 12 References

Martz RM, Reutter DJ, Lasswell LD. A comparison of ionization techniques for gas chromatography/mass spectroscopy analysis of dye and lachrymator residues from exploding bank security devices, *J Forensic Sci* 1983; 28: 200-207

Verweij AMA, Lipman PJJ. Comparison of mass spectrometric techniques for the analysis of trace amounts of 1-Methylaminoanthraquinone used as smoke dye in exploding money suitcases. *J Chromatography A* 1993; 653: 359-362

Redacted

Kataoka M, Seto Y, Tsuge K, Naomi M. Stability and detectability of lachrymators and their degradation products in evidence samples. *J Forensic Sci* 2002; 47: 44-51

Jagardeo E, et al. Analysis of trace amounts of bank dye and lachrymators from exploding bank devices by solid phase microextraction and gas chromatography-mass spectrometry. *J Chromatogr Sci* 2006; 44: 86-90

*Guidelines for Comparison of Mass Spectra*; FBI Laboratory Chemistry Unit – General Chemistry SOP

*FBI Laboratory Safety Manual*



Rev. #	Issue Date	History
2	05/26/11	Added DART to section 3. Updated section 4. Revised sections 5.2 a and b. <span style="color: red;">Redacted</span>
		Revised section 8c and 8g. Revised section 10.2 DART/TOF-MS instrument parameters to give more detail. Revised section 11.1.2f. Revised section 13e. Corrected reference in section 15.
3	01/15/20	Revised title. Removed previous section 1 (Introduction), section 3 (Principle), and section 6 (Calibration), and renumbered sections 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. Edited new section 3.1 to add detail. Changed lettered listing in section 3.2 to bullets and edited section for clarity. Detail added to new section 4 (Sampling). Section 5 edited to add more details. Removed use of ultraviolet light to observe for fluorescence. Changed previous section 9 to sections 6 and 6.1 and added detail. Removed TLC information from section 8 and revised entire section for clarity and to allow for flexibility. Minor edits to sections 9.1 and 9.3, added section 9.2. Changed title of section 10 (was 'Limitations of Procedure'); removed previous 1st bullet and added new 1 <sup>st</sup> , 2 <sup>nd</sup> , and 4 <sup>th</sup> bullets in section 10. Removed approximate LODs from Limitations sections. Updated references section (content and format).

### Approval

Redacted - Signatures on File

Chemistry Unit Chief:

Date: 01/14/2020

General Chemistry  
 Technical Leader:

Date: 01/14/2020

## Comparison of Inks

### 1 Scope

This procedure allows for the analysis of writing, marking, or other inks from various matrices (e.g., paper, cardboard, cloth). Comparisons are made between questioned and known ink items, which allow conclusions to be reached regarding the possible common origin of the inks. Identification of inks by manufacturer and dating of inks are beyond the scope of this procedure.

This procedure applies to Chemistry Unit (CU) personnel that are qualified to compare inks.

### 2 Equipment/Materials/Reagents

- Common laboratory glassware and equipment
- Stereo microscope
- Digital microscope
- Ultraviolet (UV) light source
- CrimeScope CS-16 light source
- Video Spectral Comparator (VSC)
- Whatman HP pre-coated silica gel TLC plate (10 cm x 10 cm) (or equivalent)
- TLC tank
- Blunt-tipped sampling device (e.g., core sampler)
- n-Butanol
- Deionized water
- Ethanol
- Ethyl acetate
- Methanol
- Pyridine, low water

### **3 Standards and Controls**

#### **3.1 Negative Control**

A Negative Control will be prepared from an area of the questioned item where no ink is present. If there are no unmarked areas of the item, a solvent blank will be used as a Negative Control. If there is printing on the opposite side of the item (e.g., security printing on the inside of an envelope) then the Negative Control will be sampled from a portion of the questioned item that contains the printing. It is left to the discretion of the examiner as to what constitutes an adequate Negative Control.

#### **3.2 Positive Control**

Since this procedure is based on comparative tests, positive controls are typically not applicable. However, when a known ink will be deposited on a matrix to facilitate a comparison, the matrix will be similar to the matrix of the questioned item. Blank samples from the matrix will also be sampled. Subsequent extraction of the known ink and matrix will serve as a Positive Control.

### **4 Preparation of Solvent Systems**

#### **4.1 Solvent System I**

Prepared by combining 70 mL of ethyl acetate, 35 mL of ethanol, and 30 mL of deionized water (14:7:6 volume ratio) in a beaker and mixing thoroughly. Transfer an aliquot of Solvent System I to a TLC tank and allow it to equilibrate for 15 minutes.

#### **4.2 Solvent System II**

Solvent System II is used when samples were not differentiated by Solvent System I. Solvent System II is prepared by combining 50 mL of n-butanol, 10 mL of ethanol, and 15 mL of deionized water (10:2:3 volume ratio) in a beaker and mixing thoroughly. Transfer an aliquot of Solvent System II to a TLC tank and allow it to equilibrate for 15 minutes.

## 5 Sampling

Ink samples are screened with a combination of light sources (e.g., visible, UV, CrimeScope, VSC) to ensure homogeneity before collecting plugs.

When performing this procedure on printer-related evidence (e.g., ink cartridges), ink samples obtained from printing with the device are preferred. For a full color inkjet printer, samples will include multiple punches of each available color (typically cyan, magenta, yellow, and black) when possible.

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, the results of examinations will be clearly limited to the sample(s) that were selected and analyzed.

## 6 Procedure

- a. View the ink sample and record relevant observations. Observe the sample under magnification to determine whether the writing was produced by a ballpoint or non-ballpoint device. Ballpoint pen writing will usually show signs of skipping, gooping, and burr striations. Record observations with photography or digital imaging if possible.
- b. If an item consists of a writing or printing device, prepare a Positive Control by using the device to deposit a sample of the ink onto a matrix that is similar to the matrix of the questioned item. Allow the sample to dry. Perform step (a) on the Positive Control to the extent necessary. An ink sample may be directly removed from the device if it is not possible to use the device as intended.
- c. Place the matrix (e.g., document) on a cutting mat and use a core sampler to individually remove ~1 mm diameter plugs (typically 5 to 10 plugs) from an area of the matrix which does not contain ink for use as a Negative Control. Transfer the plugs to a labeled test tube ensuring each of the plugs is transferred. If the matrix is not conducive to removing plugs with a core sampler, obtain samples via cutting or other method ensuring similar areas are obtained for all items.
- d. Perform step (c) for each area that contains an ink sample of interest, as well as any Positive Control(s) from step (b), ensuring that the same number of plugs is collected for all samples. Blank plugs from the Positive Control matrix will also be collected.
- e. Add 7 to 15 uL of solvent (e.g., pyridine, methanol) to each of the test tubes. Vortex and

allow the extracts to sit for ~2 minutes. The amount of solvent may need to be varied for non-plug samples. Dilute any ink extract in the initial solvent if the extract is substantially darker in color than the comparison solution. The goal is to obtain consistency in extract concentrations among the ink samples. Ideally, an ink extract will have a distinct color and be transparent.

- f. Record the colors of the extracts.
- g. Spot a portion of each of the extracts ~1 cm from the bottom of a TLC plate. Allow the spots to dry. If additional sample is needed, repeat the spotting process until the desired spot intensity is achieved. Typically, it is unnecessary to repeat the spotting process. The TLC plate will have sample spots from the Negative Control(s) and questioned ink sample(s), as well as any applicable Positive Control(s) and associated matrix blank(s).
- h. Record the color of all of the spots that were deposited on the TLC plate.
- i. Transfer the plate into the Solvent System I TLC tank.
- j. After ~10 minutes, remove the plate from the TLC tank and allow it to dry.
- k. Record observations [e.g., any visibly separated components, colors of the components, relative concentrations, relative distances ( $R_f$  values)]. Photographs should be taken with a ruler in the field of view.
- l. View the TLC plate under alternate light sources (e.g., UV light, CrimeScope, VSC) and record observations.
- m. If differences are observed between comparison samples (e.g., different ink component  $R_f$  values), then only Solvent System I is necessary.
- n. If additional sample extracts remain, use it to spot a new TLC plate as described in step (g) and record the color of all of the spots that were deposited on the TLC plate. Otherwise, repeat steps (c) through (h).
- o. Transfer the new plate into the Solvent System II TLC tank.
- p. Repeat steps (j) through (l) for the new plate.

## 7 Calculations

$R_f = (\text{distance spot traveled from origin}) / (\text{distance of solvent front from origin})$

## 8 Measurement Uncertainty

Not applicable

## **9 Decision Criteria**

### **9.1 Visual Observations**

#### **9.1.1 Indistinguishable Samples**

If no distinguishable characteristics are observed between the ink samples, this will be recorded and TLC will be performed.

#### **9.1.2 Differentiated Samples**

If differences are observed between ink samples by visual and/or microscopic exams, this will be recorded and no further exams are necessary. Photography or digital imaging will be used to record the differences if no further exams will be conducted.

### **9.2 TLC**

#### **9.2.1 Writing Inks**

##### **9.2.1.1 Indistinguishable Samples**

Ink samples are considered indistinguishable if all separated colorant components, relative concentrations, and  $R_f$  values correlate well between the samples in both solvent systems.

##### **9.2.1.2 Differentiated Samples**

If differences are observed in the general appearance of any separated colorant components, relative concentrations, and/or  $R_f$  values, this will be recorded and the samples will be reported as differentiated. If this occurs with Solvent System I, analysis with Solvent System II is not required.

#### **9.2.2 Inkjet Inks**

##### **9.2.2.1 Indistinguishable Samples**

Ink samples are considered indistinguishable if all separated colorant components, relative concentrations, and  $R_f$  values correlate well between the samples in both solvent systems. There may be instances when an inkjet colorant (e.g., cyan, magenta, yellow, black) is not present on an item. The absence of a colorant in a comparison should not be used to distinguish samples in these instances.

### 9.2.2.2 Differentiated Samples

If differences are observed in the general appearance of any separated colorant components, relative concentrations, and/or  $R_f$  values where comparable colors (e.g., cyan, magenta, yellow, black) were sampled, this will be recorded and the samples will be reported as differentiated. If this occurs with Solvent System I, analysis with Solvent System II is not required.

## 10 Limitations

This procedure is limited to writing inks (e.g., pens, markers) and inkjet inks. The available sample size may limit or preclude the analysis from being performed. Items that have small amounts of ink may not be able to be analyzed by this procedure. Certain matrices (e.g., plastics, clothing) may hinder or prevent adequate extraction and/or separation of the ink components. The following conclusions apply to the comparison of inks:

- Cannot be differentiated
- Excluded
- 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.

## 11 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.

## 12 References

ASTM E1422-05 – Standard guide for test methods for forensic writing ink comparison (Withdrawn 2014).

ASTM E1789-04 – Standard guide for writing ink identification (Withdrawn 2013).

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Weyermann, C, Marquis, R, Mazzella, WM, Spengler, B. *J Forensic Sci* 2007; 52(1): 216-220.

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

*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)

*FBI Laboratory Safety Manual*



Rev. #	Issue Date	History
2	04/01/11	Updated title. Reworded sections 1, 3, 5.2, and 8 for clarity. Removed all materials associated with capillary electrophoresis (CE) from section 4, as well as from within the rest of the document. Added ALS and blunt-tipped device to section 4. Removed magnifying glass from section 4b. Added statements to section 5.1 and 5.2 regarding Negative and Positive Control sampling for clarity. Included photo documentation in section 8b. Removed specific requirements from, and added TLC tank to, section 10.1. Photography was added to section 11.1. Added visual observations to section 11.1. The TLC decision criteria was moved to section 11.2 and was re-worded. A statement regarding other types of inks was added to section 13. Updated and re-worded references.
3	05/04/20	Removed previous sections 1 (Introduction), 3 (Principle), 6 (Calibration), and 10 (Instrumental Conditions); renumbered sections accordingly. Edited new section 1 for clarity and to include personnel. Changed lettered listing in new section 2 to bullets and revised the list. Minor edits to sections 3.1 and 3.2. Added new section 4 (was contained within procedure section). Added significant detail to section 5. Section 6 was edited throughout for clarity and flexibility. Minor edit made to section 7 for clarity. Changed new section 8 title from 'Uncertainty of Measurement'. Changed formatting, made minor edits, and added content covering inkjet inks to section 9. Changed formatting, made minor edits, and added content including conclusions and references to ASSTR, ULTR, etc. to section 10. Updated references in section 11.

### **Approval**

Redacted - Signatures on File

Chemistry Unit Chief:

Date: 05/01/2020

General Chemistry  
Technical Leader:

Date: 05/01/2020

## **Analysis of Lubricants, Waxes, Oils, and Related Compounds**

### **1 Scope**

This procedure allows for the analysis of a wide variety of lubricant materials (e.g., petroleum products, waxes, oils) that may be relevant to many types of investigations (e.g., sexual assault, drug trafficking, vehicular hit-and-run). Identification of a specific substance is not always possible, however a general classification is typically achievable.

This procedure applies to Chemistry Unit (CU) personnel that are qualified to examine evidence for the presence of lubricants, oils, waxes, and related compounds.

### **2 Equipment/Materials/Reagents**

- Common laboratory glassware and equipment
- Analytical balance
- Stereo microscope
- Polarized Light Microscope (PLM)
- Digital microscope (includes PLM capability)
- Ultraviolet (UV) light source
- CrimeScope CS-16 light source
- Video Spectral Comparator (VSC)
- Evaporator
- Fourier Transform Infrared (FTIR) spectrophotometer with Attenuated Total Reflectance (ATR), transmission, or microscope attachments
- High temperature gas chromatography with flame ionization detection (GC-FID) equipped with a 15 meter Zebron "Inferno" ZB-1HT column (or equivalent)
- Gas chromatograph/mass spectrometer (GC/MS) equipped with an electron impact ionization source and a 30 meter DB-5 column (or equivalent)
- GC/MS equipped with a chemical ionization source and a 30 meter DB-5 column (or equivalent)
- Time-of-flight mass spectrometer with direct analysis in real time ionization source (DART/TOFMS)
- Scanning electron microscope with energy dispersive X-ray spectrometer (SEM/EDS)
- Chloroform
- Deionized water
- Hexane

- Iodine
- Methanol
- Nonoxynol-9 (Ipegal®CO-630)
- Polydimethylsiloxane (PDMS, Dimethylpolysiloxane)
- Polyethylene glycol (PEG, 550 average molecular weight)
- Potassium Iodide
- Starch
- Trichloroethane

### **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 solvent from the same source and lot and within a similar container used to extract or dissolve a questioned item(s). If swabs are submitted as evidence, a blank swab (preferably from the same source as the evidence swabs) extracted in the same manner as the questioned item(s) will be used as a Negative Control. In some instances, an extract from a portion of the item(s) which does not contain a stain of interest may be selected for use as a Negative Control. It is left to the discretion of the examiner as to what constitutes an adequate Negative Control.

#### **3.2 Positive Control**

A Positive Control will be prepared from an appropriate reference or known material. When appropriate the Positive Control will be prepared within a matrix that best mimics the questioned item(s). Similarly, where relevant the concentration of the Positive Control will be prepared to mimic the questioned item(s). It is left to the discretion of the examiner as to what constitutes an adequate Positive Control.

### **4 Preparation of Potassium Iodide/Iodine Color Test Reagent**

The Potassium Iodide/Iodine Working Solution will be verified at the time of use through the testing of Negative and Positive Controls. The amounts of materials indicated in this section may be scaled up or down as necessary.

#### **4.1 Potassium Iodide/Iodine Stock Solution**

Prepared by adding 6 grams of potassium iodide and 0.8 grams of iodine crystals to 100 mL of deionized water. Store the solution at room temperature in a brown/amber colored glass bottle.

#### **4.2 Potassium Iodide/Iodine Working Solution**

Prepared by diluting the potassium iodide/iodine stock solution 1:100 with deionized water. Store the solution at room temperature in a brown/amber colored glass bottle.

### **5 Sampling**

Typically, one or more samples (e.g., cuttings) are selected from the stained area(s) of the questioned item. When multiple samples are selected from the same item, the samples are typically combined prior to extraction.

Multiple items that are packaged together (e.g., swabs) or otherwise in contact with each other will typically be sampled as one collective item. For example, if two swabs are packaged together, one swab (or a portion of the swab) will typically be sampled as representative of the swabs. Multiple swabs may be sampled and extracted together if the staining appears to be minimal.

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, the results of examinations will be clearly limited to the sample(s) that were selected and analyzed.

## 6 Procedure

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

### 6.1 General Lubricants Analysis

- a. Perform a visual and/or microscopic examination and note any distinguishing characteristics about the item. Items with no readily visible stain/substance will be analyzed using alternate light sources (e.g., CrimeScope, UV light, VSC). Any stain/substance that is subsequently visualized will be documented by photography or digital imaging, if possible.
- b. If possible, directly sample any questioned substance from the substrate with a non-porous utensil (e.g., spatula, tweezers) and transfer to a labeled test tube. Use an empty, labeled test tube as a Negative Control. Analyze the directly sampled substance by FTIR. If there are no other samples to collect skip to step (i).
- c. If the stain/substance cannot be directly sampled, take a small cutting and transfer it to a labeled test tube. Use an empty, labeled test tube as a Negative Control.
- d. If cutting is not practical, sample the area with a swab (dry or wetted with an appropriate solvent) and transfer the swab to a labeled test tube. Prepare an appropriate Negative Control swab and transfer it to an empty, labeled test tube.
- e. Extract any cuttings and/or swabs and the associated Negative Control(s) with a solvent such as hexane, trichloroethane, or MeOH:CHCl<sub>3</sub> (1:1).
- f. Transfer the Negative Control(s) and extract(s) solutions to new, labeled test tubes. Save the original test tubes for section 6.2 below.
- g. Centrifuge the Negative Control(s) and extract(s) solutions if any particulate matter needs to be removed. Decant and/or filter the Negative Control(s) and extract(s) solutions as necessary. Any solids may be analyzed by steps (e), (f), and/or (g) in section 6.2, as deemed necessary.
- h. Analyze the Negative Control(s) and extract(s) solutions by FTIR by evaporating 2 or 3 drops of the solution directly onto the ATR accessory. Ensure the ATR accessory is contamination-free prior to analyzing each sample by evaporating 2 or 3 drops of blank solvent (the same solvent and lot used to prepare the extracts) on the ATR accessory and recording the FTIR spectrum. The ATR accessory may need to be cleaned multiple times for the blank solvent to result in a blank spectrum. The extract(s) may need to be concentrated and then re-analyzed if the FTIR spectrum is too weak. If the extract(s) is concentrated, the associated Negative Control(s) will also be concentrated in a similar manner and re-analyzed by FTIR. When finished with FTIR skip to step (i).
- i. Extract any directly sampled substance(s) and the associated Negative Control(s) with an appropriate solvent such as hexane, trichloroethane, or MeOH:CHCl<sub>3</sub> (1:1).

- j. Transfer the Negative Control(s) and extract(s) solutions to new, labeled test tubes. Save the original test tubes for section 6.2 below.
- k. Centrifuge the Negative Control(s) and extract(s) solutions if any particulate matter needs to be removed. Decant and/or filter the Negative Control(s) and extract(s) solutions as necessary. Any solids may be analyzed by steps (e), (f), and/or (g) in section 6.2, as deemed necessary.
- l. Analyze the Negative Control(s) and extract(s) solutions by high temperature GC-FID. When possible, similar amounts of questioned and known samples will be analyzed for comparison purposes. For most compounds, a solution of ~0.001% to 0.01% provides an adequate signal, however some compounds (e.g., PDMS, PEG) may require higher concentrations (e.g., ~1% to 2%).
- m. For comparisons, if a questioned sample was not differentiated from a known sample by high temperature GC-FID, then GC/MS (EI and/or CI) will be used. However, if samples are suspected to contain PDMS or PEG (or other incompatible substances), do not analyze by GC/MS.
- n. Elemental analysis (e.g., SEM/EDS) may be employed as necessary on directly sampled substances. For example, some greases may contain metallic soaps (e.g., aluminum, sodium, or calcium stearates).

## 6.2 Analysis for Water Soluble Substances

- a. Allow the above Negative Control(s) and extract(s) test tubes from step (f) and/or (j) to air dry.
- b. Extract the Negative Control(s) and extract(s) with a minimal amount of deionized water to dissolve any water soluble substances that may be present (e.g., nonoxynol-9, glycerin, starch).
- c. Centrifuge the aqueous Negative Control(s) and extract(s) if any particulate matter needs to be removed. Decant and/or filter the Negative Control(s) and aqueous extract(s) as deemed necessary.
- d. Analyze the aqueous Negative Control(s) and extract(s) by DART/TOFMS in positive ionization mode. Negative ionization mode may also be used as deemed necessary.
- e. The aqueous Negative Control(s) and extract(s) may be analyzed by PLM for indications of starch. Transfer 2 or 3 drops of each onto separate glass slides with cover slips and look for the presence of Maltese crosses. Any positive results will be recorded.
- f. If a positive result for starch is obtained by PLM, the glass slides may be processed with Potassium Iodide/Iodine Working Solution. Place 1-2 drops of the Potassium Iodide/Iodine Working Solution onto the aqueous Negative Control(s) and extract(s) slides and observe under a stereo or digital microscope. The presence of small blue/purple particles indicates the presence of starch. Any positive results will be recorded.

- g. The residue from dried aqueous Negative Control(s) and extracts may be analyzed by SEM/EDS and/or FTIR for the presence of talc, silica, or other related components.

## 7 Calculations

Not applicable

## 8 Measurement Uncertainty

Not applicable

## 9 Limitations

The physical nature of the sample (e.g., sample amount, matrix) may limit or preclude some techniques from being performed. It is not always possible to identify a lubricant, however classification is typically achievable. The following conclusions apply to the analysis of lubricants and/or comparisons involving lubricants:

- Identification (i.e. identified)
- Consistent with
- Not identified
- Cannot be differentiated
- Excluded
- 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.

## 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.

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

*General Chemistry Instrumental 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 (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)

*FBI Laboratory Safety Manual*

Rev. #	Issue Date	History
2	09/13/11	Renamed title. Reworded sections 1-3 to provide further details regarding various types of lubricants and waxes and include additional instrumental techniques which may be used in the analysis. Updated section 4 regarding HT-GC(FID) column information and grade of hexanes. Revised color test reagent descriptions in section 5. Updated the description of controls in section 6. Reworded and revised section 9 for clarification. Updated instrument parameters in section 11. Reworded section 12.1.1 and section 12.1.3 for clarification.
3	05/04/20	Removed previous sections 1 (Introduction), 3 (Principle), 7 (Calibration), 11 (Instrumental Conditions), and 12 (Decision Criteria); renumbered sections accordingly. Edited new section 1 for clarity and to include personnel. Changed lettered listing in new section 2 to bullets and revised the list. Edited new sections 3.1 and 3.2 to add detail and deleted sub-sections that addressed PDMS, PEG, Nonoxynol-9, and Starch. Edited new section 4 title and content for clarity and to require reagent verification with each use (typical practice). Added detail to section 5. Renamed sub-section headings in new section 6 (was 'Analysis of Substances' and 'Analysis of Sexual Lubricant Substances') and edited content for clarity. Changed new section 8 title from 'Uncertainty of Measurement'. Added section 9. Updated references in section 11.

**Approval**

Redacted - Signatures on File

Chemistry Unit Chief:

Date: 05/01/2020

General Chemistry  
 Technical Leader:

Date: 05/01/2020

## **Analysis of Oleoresin Capsicum (OC) Sprays**

### **1 Scope**

This procedure allows for the analysis of items suspected of having been exposed to oleoresin capsicum (OC) sprays. Typical items for analysis include the contents of OC spray canisters, clothing, swabs, or any item which may have been in contact with an OC spray.

This procedure applies to Chemistry Unit (CU) personnel that are qualified to examine evidence for the presence of OC spray chemicals.

### **2 Equipment/Materials/Reagents**

- Common laboratory glassware and equipment
- Analytical balance
- Ultraviolet (UV) light source
- CrimeScope CS-16 light source
- Evaporator
- 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)
- GC/MS equipped with a chemical ionization source and a 30 meter DB-5 column (or equivalent)
- Liquid chromatography system with C18 column (or equivalent) with electrospray ionization (ESI) (e.g., Thermo LTQ, Thermo LTQ OrbiTrap XL, Thermo Exactive OrbiTrap)
- Capsaicin
- 2-Chloroacetophenone (CN tear gas)
- Deionized water
- Dihydrocapsaicin
- Formic acid
- Methanol
- Orthochlorobenzalmalononitrile (CS tear gas)
- Polyethylene glycol (PEG, 550 average molecular weight)

### **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 a questioned item(s). If swabs are submitted as evidence, a blank swab (preferably from the same source as the evidence swabs) extracted in the same manner as the questioned item(s) will be used as a Negative Control. In some instances, an extract from a portion of the item(s) which does not contain a stain of interest may be selected for use as a Negative Control. 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 Capsaicin Positive Control (100 ug/mL)**

A capsaicin stock solution (1 mg/mL) is prepared by dissolving 10 mg of capsaicin in 10 mL of methanol. The capsaicin stock solution is then diluted 1:10 with methanol to prepare the capsaicin positive control solution. Store the solutions in glass containers in a freezer.

##### **3.2.2 Dihydrocapsaicin Positive Control (100 ug/mL)**

A dihydrocapsaicin stock solution (1 mg/mL) is prepared by dissolving 10 mg of dihydrocapsaicin in 10 mL of methanol. The dihydrocapsaicin stock solution is then diluted 1:10 with methanol to prepare the dihydrocapsaicin positive control solution. Store the solutions in glass containers in a freezer.

##### **3.2.3 Capsaicin/Dihydrocapsaicin Positive Control (100 ug/mL each)**

Prepared by combining aliquots of the capsaicin and dihydrocapsaicin stock solutions and diluting 1:10 with methanol. For example, mix 1 mL of capsaicin stock solution, 1 mL of dihydrocapsaicin stock solution, and 8 mL of methanol. Store the solution in a glass container in a freezer.

### **3.2.4 Orthochlorobenzalmalononitrile (CS Tear Gas) Positive Control (50 ug/mL)**

Prepared by dissolving 5 mg of CS tear gas in 100 mL of methanol or acetone. Store the solution in a glass container in a freezer.

Alternatively, the MAAQ/CS (50 ug/mL) Positive Control from *Analysis of Bank Security Device Chemicals* (GenChem 2) may be used as a CS tear gas Positive Control.

### **3.2.5 2-Chloroacetophenone (CN Tear Gas) Positive Control (50 ug/mL)**

Prepared by dissolving 5 mg of 2-chloroacetophenone in 100 mL of methanol or acetone. Store the solution in a glass container in a freezer.

## **4 Sampling**

Typically, one or more samples (e.g., cuttings) are selected from the stained area(s) of the questioned item. When multiple samples are selected from the same item, the samples are typically combined prior to extraction.

Multiple items that are packaged together (e.g., swabs) or otherwise in contact with each other will typically be sampled as one collective item. For example, if two swabs are packaged together, one swab (or a portion of the swab) will typically be sampled as representative of the swabs. However, multiple swabs may be sampled and extracted together if the staining appears to be minimal.

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, the results of examinations will be clearly limited to the sample(s) that were selected and analyzed.

## 5 Procedure

- a. Perform a thorough visual examination of the item for the presence of stains. Record the color and location of any observed stains. The nozzle of a submitted OC spray canister will be inspected for the presence of stains. Items with no readily visible stains will be analyzed using the CrimeScope and UV light sources. Any stains that are subsequently visualized will be documented by photography, if possible.
- b. Cut a small section ( $\sim 1 \text{ cm}^2$ ) from the stained area of the item and transfer the cutting to a labeled test tube. Multiple cuttings from the same item, or items that were packaged together, may be combined. Use an empty, labeled test tube as a Negative Control.
- c. If cutting is not practical, sample the area with a swab wetted with methanol and transfer the swab to a labeled test tube. Prepare a Negative Control swab wetted with methanol and transfer it to an empty, labeled test tube.
- d. Add enough methanol (typically 1 to 2 mL) to the cuttings and/or swabs to submerge the sample(s). Use the same volume of methanol for the Negative Control(s) and each of the sample(s). Vortex mix then extract the Negative Control(s) and sample(s) for  $\sim 5$  minutes by rotation/inversion.
- e. Transfer the Negative Control(s) and extract(s) solutions to new, labeled test tubes. Filter any solutions that contain particulates with 0.2  $\mu\text{m}$  PTFE syringe filters that have been pre-rinsed with methanol. Also filter the applicable Negative Control(s). Collect the filtrates in new, labeled test tubes.
- f. Concentrate the Negative Control(s) and extract(s) solutions under  $\text{N}_2$  (g) flow at  $\sim 60^\circ\text{C}$  to a final volume of  $\sim 150$  to  $200 \text{ uL}$ . Do not allow the solutions to go to dryness as CS or CN tear gas residues may be lost.
- g. If an OC spray canister was submitted as a comparison sample, obtain a sample of the contents by briefly spraying the canister into a wide mouthed glass or plastic container while in a fume hood. Use an empty, labeled container from the same supply as a Negative Control. Transfer an aliquot of the OC spray contents to a labeled test tube and prepare a dilution of  $\sim 1\%$  in methanol. Add the same volume of methanol that was used to dilute the OC spray to the Negative Control container and sample the interior, then transfer the methanol to a labeled test tube.
- h. Analyze the Negative Control(s), questioned item(s), and Positive Control(s) solutions by DART/TOFMS. Sample each solution with the closed end of a glass capillary. PEG will be analyzed with each data collection file to allow for mass-to-charge correction.
- i. Analyze the Negative Control(s), questioned item(s), and Positive Control(s) solutions by GC/MS using electron impact (EI) ionization mode. Incorporate methanol blanks between all samples. Splitless injection mode may be necessary for weakly concentrated solutions.
- j. If interferences for capsaicin and/or dihydrocapsaicin were observed by GC/MS and could not be mitigated with extracted ion chromatograms, analyze the applicable

Negative Control(s), questioned item(s), and Positive Control(s) solutions by LC/MS (ESI). Incorporate methanol blanks between all samples.

- k. If CS tear gas is suspected, analyze the applicable Negative Control(s), questioned item(s), and Positive Control(s) solutions by GC/MS using negative ion chemical ionization (NICI) mode. Incorporate methanol blanks between all samples. [Note- GC/MS (NICI) should be performed even if analysis by DART/TOFMS and/or GC/MS (EI) was negative for CS tear gas since GC/MS (NICI) is more sensitive for CS tear gas.]
- l. If CN tear gas is suspected, analyze the applicable Negative Control(s), questioned item(s), and Positive Control(s) solutions by GC/MS using positive ion chemical ionization (PICI) mode. Incorporate methanol blanks between all samples. [Note- GC/MS (PICI) should be performed even if analysis by DART/TOFMS and/or GC/MS (EI) was negative for CN tear gas since GC/MS (PICI) is more sensitive for CN tear gas.]

## 6 Calculations

Not applicable

## 7 Measurement Uncertainty

Not applicable

## 8 Instrumental Conditions

Refer to *General Chemistry Instrumental 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: 0.05% v/v formic acid (aq)		total flow = 0.35 mL/min			type	C18
		time (min)	% A	% B	length	150 mm
B: Methanol		0	40	60	internal diameter	2.1 mm
		0.5	40	60	particle size	5 um
		5.5	10	90	temperature	30 °C
Autosampler		6.5	10	90		
temperature	15 °C	6.6	40	60		
injection volume	10 uL	total run time = 11 min.				



### 8.1.2 Mass Spectrometer Parameters

Duration = 9.00 min; 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.	
<b>Scan Event #1</b>	
<b>Ionization mode</b>	ESI (+)
<b>Scan mode</b>	Full scan MS
<b>Scan range</b>	150-550 <i>m/z</i>
<b>Scan Event #2</b>	
<b>Ionization mode</b>	ESI (+)
<b>Scan mode</b>	Product ion MS/MS
<b>Precursor ion</b>	306.7
<b>Collision energy</b>	11 %
<b>Product scan range</b>	80-340 <i>m/z</i>
<b>Q</b>	0.250
<b>Time</b>	30.000
<b>IsoW</b>	2.0
<b>Scan Event #3</b>	
<b>Ionization mode</b>	ESI (+)
<b>Scan mode</b>	Product ion MS/MS
<b>Precursor ion</b>	308.7
<b>Collision energy</b>	10 %
<b>Product scan range</b>	80-340 <i>m/z</i>
<b>Q</b>	0.250
<b>Time</b>	30.000
<b>IsoW</b>	2.0

## 9 Limitations

This procedure is primarily used for items that have visible stains (or stains that can be visualized using alternate light sources). The absence of visible stains on items may prevent the identification of capsaicinoids. Certain matrices may minimize the absorption of OC sprays upon exposure (e.g., Gore-Tex, nylon) or may interfere with the extraction and/or identification of OC sprays (e.g., plastics, heavily-stained items). The following conclusions apply to the analysis of OC sprays and/or comparisons involving OC sprays:

- Identification (i.e. identified)
- Consistent with

- Not identified
- Cannot be differentiated
- Excluded
- 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.

## 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.

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

*General Chemistry Instrumental 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 (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)

*FBI Laboratory Safety Manual*

Rev. #	Issue Date	History
2	01/11/11	Added CS tear gas to section 4. Added instructions how to prepare CS tear gas positive control to section 5.2. Added statement regarding CS tear gas to section 8i. Added NICI mass range to section 9.1.
3	05/04/20	Removed previous sections 1 (Introduction), 3 (Principle), 6 (Calibration), and 10 (Decision Criteria); added section 6 (Calculations); renumbered sections accordingly. Edited new section 1 for clarity and to include personnel; defined "CU". Changed lettered listing in new section 2 to bullets and revised the list. Edited new sections 3.1 and 3.2 to add detail; changed formatting; added CN tear gas and reference to MAAQ/CS positive control. Added content to section 4 (Sampling). Edited content of section 5 for clarity and added DART/TOFMS, GC/MS (PICI), and LC/MS (ESI) as instrumental techniques. Changed new section 7 title from 'Uncertainty of Measurement'. Section 8 edited to refer to GenChem 34 and added LC/MS (ESI) parameters. Section 9 edited to include conclusion statements and references to ASSTR, ULTR, etc. Updated references in section 11.

**Approval**

Redacted - Signatures on File

Chemistry Unit Chief:

Date: 05/01/2020

General Chemistry  
 Technical Leader:

Date: 05/01/2020

# VALIDATION SUMMARY

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## CHEMISTRY UNIT // FBI Laboratory

**Procedure Name:** Gen Chem SOP 5-2 (Supplement by adding LC/MS/MS as an analytical tool for capsaicin and dihydrocapsaicin)

**Date:** April 15, 2019

### **Validation Summary:**

Chromatographic, ionization, and mass spectrum conditions were optimized in order to detect capsaicin and dihydrocapsaicin by LC/MS/MS. The LOD for both capsaicin and dihydrocapsaicin was determined to be equal to or less than 0.5 ug/mL. No interferences were identified from the various matrices evaluated.

### **Approval:**

Technical Leader: \_\_\_\_\_ Date: \_\_\_\_\_

Chemistry Unit Chief: \_\_\_\_\_ Date: \_\_\_\_\_

## Drug Analysis

### 1 Scope

This procedure is used for the analysis of items suspected of containing drugs. Questioned items may consist of a variety of substances including unknown solids and liquids, prescription medications, and over-the-counter products [see *Analysis of Tablets and Capsules* (GenChem 7) for prescription and over-the-counter tablets, capsules, etc.]. While the below procedure describes many techniques, identification of a drug relies upon positive results from two orthogonal techniques with at least one of the techniques providing structural elucidation information.

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

### 2 Equipment/Materials/Reagents

- Common laboratory glassware and equipment
- Analytical balance
- Digital microscope
- Stereo microscope
- Ultraviolet light source (long wavelength)
- Acetaldehyde
- Acetonitrile
- Ammonium hydroxide
- Chloroform
- Cobalt thiocyanate
- Deionized water
- Diethyl ether
- Formaldehyde (40%)
- Hydrochloric acid
- Methanol (MeOH)
- Nitric acid
- Sodium bicarbonate
- Sodium carbonate
- Sodium nitroprusside (aka sodium nitroferricyanide)
- Sulfuric acid

- Evaporator
- Fourier Transform Infrared (FTIR) spectrophotometer with Attenuated Total Reflectance (ATR) or microscope attachment
- Polyethylene glycol (PEG, 550 average molecular weight)
- 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)
- Gas chromatograph/mass spectrometer (GC/MS) equipped with chemical ionization and a 30 meter DB-5 column (or equivalent)

### **3 Standards and Controls**

#### **3.1 Negative Control**

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

#### **3.2 Positive Control**

Prepared by making a 1 mg/mL (as base) stock standard solution of the drug into a suitable solvent. A working standard solution of 0.1 mg/mL is typically used with GC/MS by diluting the stock standard 1:10. These solutions will be stored in a freezer or refrigerator. Other concentrations may be prepared and used as needed.

### **4 Preparation of Color Test Reagents**

#### **4.1 Marquis Reagent**

Prepared by adding 8-10 drops of 40% formaldehyde to 10 mL of concentrated sulfuric acid. This solution is stored at room temperature in an amber glass bottle. Discard the solution when it begins to discolor.

## 4.2 Scott's Reagent

- Reagent A- Prepared by adding 2 grams of cobalt thiocyanate to 100 mL of deionized water. Mix thoroughly. The solution should be pink in color. This solution is stored in a glass bottle at room temperature.
- Reagent B- Prepared by adding 8.5 mL of concentrated hydrochloric acid to 80 mL of deionized water and then diluting to 100 mL with deionized water. This solution is stored in a glass bottle at room temperature.

## 4.3 Sodium Nitroprusside Reagent

- Reagent A- Prepared by dissolving 1.1 grams of sodium nitroprusside (aka sodium nitroferrocyanide) into 100 mL of deionized water and 4 mL of acetaldehyde. This solution is stored in an amber glass bottle in a refrigerator.
- Reagent B- Prepared fresh by dissolving 2 grams of sodium carbonate in 100 mL of deionized water. A small amount of solid sodium carbonate (or sodium bicarbonate) can be used in lieu of the aqueous solution.

## 5 Sampling

Sampling is performed according to the *Sampling Guidelines for Bulk Materials and Multi-Unit Populations*– General Chemistry SOP manual.

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

## 6 Procedure

- a. Use a traceable analytical balance to record the weight for each item, as applicable.
- b. Perform a visual examination of each item. Microscopy may be used as deemed necessary. Suspected lysergic acid diethylamide (LSD) samples may be observed under long wavelength ultraviolet light for blue fluorescence.
- c. Color tests may be performed. A spot plate should be used and all samples (e.g., controls, items) will be added to the reagent to ensure that the spot plate well is free of contamination. Color tests not described below may be prepared and used following similar practices. Include a copy of the reference relied upon for the color test. Examples of resources include *Clarke's*



*Analysis of Drugs and Poisons, SWGDRUG Drug Monographs, and the DEA Analysis of Drugs Manual.*

Marquis Test:

- Add 2-3 drops of Marquis Reagent to the required number of spot plate wells. Add samples to separate wells and observe and record any changes. One well will remain unaltered during the exam to demonstrate the Marquis Reagent does not change color spontaneously. A purple color indicates the possible presence of an opiate. A violet to black color indicates the possible presence of 3,4-methylenedioxyamphetamine (MDA) or 3,4-methylenedioxymethamphetamine (MDMA). An orange color indicates the possible presence of an amphetamine compound.

Nitroprusside Test:

- Add 2-3 drops of Sodium Nitroprusside Reagent A to the required number of spot plate wells. Add samples to separate wells and observe and record any changes. Next, add 2-3 drops of Sodium Nitroprusside Reagent B (or a small amount of solid sodium carbonate or sodium bicarbonate) to each well and observe and record any changes. One well will only have Reagents A and B added to it to demonstrate that a color change does not occur. A blue or violet color upon addition of Reagent B indicates the possible presence of an amphetamine compound.

Scott's Test:

- Add 2-3 drops of Scott's Reagent A to the required number of spot plate wells. Add samples to separate wells and observe and record any changes. Next, add 2-3 drops of Scott's Reagent B to each well and observe and record any changes. One well will only have Reagents A and B added to it to demonstrate that a color change does not occur. A blue color prior to the addition of Reagent B indicates the possible presence of cocaine hydrochloride, whereas a blue color after the addition of Reagent B indicates the possible presence of cocaine base.

Concentrated Nitric Acid Test:

- Add 2-3 drops of concentrated nitric acid to the required number of spot plate wells. Add samples to separate wells and observe and record any changes. One well will remain unaltered during the exam to demonstrate the nitric acid does not change color spontaneously. An orange color that fumes indicates the possible presence of acetaminophen. An orange color that does not fume indicates the possible presence of

morphine or codeine. A lime green color indicates the possible presence of guaifenesin or methocarbamol. A blue fluorescence under ultraviolet light indicates the possible presence of quinine.

- d. A representative sample may be analyzed by FTIR-ATR. A solid/powder item may be homogenized using a mortar and pestle. Liquid samples may be analyzed neat and/or allowed to evaporate on the ATR cell. The FTIR microscope attachment may be used as appropriate.
- e. If the item is a mixture, the following basic sequential solvent extraction may be used to isolate individual compounds for further analysis. Most basic organic drugs will be soluble in diethyl ether, while most drug salts will be soluble in chloroform. Sugars will usually be soluble in methanol. Utilize a fume hood for this process.
  - Homogenize a representative portion of the item with a mortar and pestle.
  - Place a piece of folded filter paper into a funnel and place the funnel over an evaporating dish. Place the homogenized powder in the filter paper.
  - Pour 2 to 3 mL of diethyl ether over the powder and collect the filtrate into the evaporating dish. Set the dish to the side and allow the diethyl ether extract to evaporate.
  - Place a new evaporating dish under the funnel and wash any remaining powder with 2 to 3 mL of chloroform. Set the dish to the side and allow the chloroform extract to evaporate.
  - Place a new evaporating dish under the funnel and wash any remaining powder with 2 to 3 mL of methanol. Allow the methanol extract to evaporate.
  - Any remaining solids in the filter paper, as well as any solids recovered from the evaporated extracts may be analyzed by FTIR-ATR.
- f. An appropriate amount of the item may be placed into a small test tube and extracted or dissolved in an appropriate solvent (e.g., methanol, chloroform, acetonitrile) to achieve the desired concentration. For example, a concentration of ~1 mg/mL may be desirable for DART/TOFMS analysis, while a concentration of ~100 ug/mL is common for GC/MS analysis. If necessary, filter the solution or centrifuge and decant to remove any undissolved particulates. The Negative Control will be filtered or centrifuged as well.
- g. If the item is an empty syringe (or similar item), rinse the interior of the barrel with a volume of an appropriate solvent (e.g., methanol, chloroform, acetonitrile) that is approximately equivalent to the volume capacity of the barrel. If the needle is intact and needs to be preserved for future DNA exams, then remove the plunger (if present) to introduce the rinse solvent to the barrel. Use the rinse to sample the barrel multiple times, then transfer the rinse to a labeled test tube. If necessary, concentrate the rinse and Negative Control under N<sub>2</sub> (g) at ~60 °C.

- h. The solution from (f.) or (g.) may be analyzed by color tests [see step (c.)]. A Negative Control will be analyzed along with the item extract(s).
- i. The solution from (f.) or (g.) may be analyzed by DART/TOFMS in positive and/or negative ionization mode (as appropriate based on the target analyte) by sampling the solution with the closed end of a glass capillary. Analyze the Negative Control(s), the Positive Control(s) (if applicable at this point), and PEG within the same data collection file. If the Positive Control(s) is determined after the initial DART/TOFMS analysis, then analyze the Positive Control(s) and PEG within a separate data collection file. It is also acceptable to analyze an item prior to extraction/dilution. For powder samples, a glass capillary can be wetted with deionized water and then touched to the sample; collect a blank glass capillary wetted with deionized water as a Negative Control.
- j. The solution from (f.) or (g.) may be analyzed by GC/MS in the electron impact (EI) mode. Also analyze the Negative Control and Positive Control(s) (if applicable at this point), and incorporate a solvent blank between each sample. If the Positive Control(s) is determined after the initial GC/MS analysis, then analyze the Positive Control(s) within a separate sequence.
- k. The solution from (f.) or (g.) may be analyzed by GC/MS in the positive ion chemical ionization (PICI) or negative ion chemical ionization (NICI) mode as appropriate. Also analyze the Negative Control and a Positive Control (if applicable at this point), and incorporate a solvent blank between each sample. If the Positive Control(s) is determined after the initial GC/MS analysis, then analyze the Positive Control(s) within a separate sequence. If an amphetamine (or other drug which gives a limited EI spectrum) is suspected, and FTIR or DART/TOFMS has not been utilized (or did not provide sufficient information), then PICI will be performed. A basic extraction using sodium bicarbonate and chloroform may improve the chromatography of methamphetamine.
- l. Other analytical techniques not listed above may be used to analyze a drug as deemed necessary {e.g., X-ray Powder Diffractometry (XRD), Liquid Chromatography/Mass Spectrometry [LC/MS, with electrospray (ESI) or atmospheric-pressure chemical ionization (APCI)], Raman spectrophotometry, Ultraviolet-Visible (UV-Vis) spectroscopy, Thin-Layer Chromatography (TLC)} provided that the instrumental conditions are retained in the case notes, the Negative Control and Positive Control samples provide the appropriate responses, and any relied upon references are retained in the case notes. These techniques are reserved for instances when the preceding steps don't provide sufficient data to identify a drug. If it is anticipated that the technique will be used routinely in the future for the drug, then the technique will be validated per the *Chemistry Unit Validation of Analytical Procedures* (CU QAOM 11).

## 7 Calculations

Not applicable.

## 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 on the CU s:\ drive.

## 9 Instrumental Conditions

The following instrumental conditions are typically used for drug analysis. Minor modifications to the conditions may be used as needed and without authorization, provided the same conditions are used for applicable solvent blanks, Negative Control(s), questioned items, and Positive Control(s); and the Positive Control(s) provide acceptable data. The utilized conditions will be retained with the case notes. [Note- some of the parameters are unique to an individual instrument and will be different among similar instruments (e.g., tune parameters).]

### 9.1 FTIR

#### 9.1.1 Benchtop with ATR

- Scan range: 4000-400  $\text{cm}^{-1}$
- Scans: 32 (sample and background scans)
- Resolution: 4  $\text{cm}^{-1}$

#### 9.1.2 Microscope

- Scan range: 4000-650  $\text{cm}^{-1}$
- Scans: 128
- Resolution: 4  $\text{cm}^{-1}$

## 9.2 DART/TOFMS

DART Source Settings	
Polarity	Positive or Negative
Source gas	Helium
Temperature	400 °C

TOFMS Settings	
Polarity	Positive or Negative
Ion Guide RF (or equivalent)	800 V for 80-800 $m/z$ , 500 V for 50-500 $m/z$

## 9.3 Gas Chromatography/Mass Spectrometry (GC/MS)

### 9.3.1 Electron Impact (EI)

GC Settings	
Injection Mode	Split or Splitless (sample dependent; consistent across blanks, controls, samples)
Injection Volume	1 uL
Inlet Temperature	250 °C
Oven Program	60 °C for 2 min, 35 °C /min to 260 °C for 15 min [some analytes require longer final times and/or higher final temperatures (e.g., steroids, heavier analytes)]
MS Settings	
Polarity	Positive
Scan Range	43 to 400 $m/z$ (increase final $m/z$ for heavier target analytes to 50 $m/z$ greater than molecular ion)

### 9.3.2 Chemical Ionization (CI)

GC Settings	
Injection Mode	Split or Splitless (sample dependent; consistent across blanks, controls, samples)
Injection Volume	1 uL
Inlet Temperature	250 °C
Oven Program	60 °C for 2 min, 35 °C /min to 260 °C for 15 min [some analytes require longer final times and/or higher final temperatures (e.g., steroids, heavier analytes)]

MS Settings	
Polarity	Positive or Negative
Reagent Gas	Methane
Scan Range	100 to 500 $m/z$ (increase final $m/z$ for heavier target analytes to 50 $m/z$ greater than molecular ion)

## 10 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of data produced in this procedure.

### 10.1 FTIR

- The FTIR spectrum for the analyte of interest should compare favorably to the corresponding Positive Control (or library) spectrum.
- If FTIR is the only instrumental technique used, a Positive Control of the analyte of interest must be contemporaneously analyzed on the same instrument.

### 10.2 DART/TOFMS

Peaks of interest should be within  $\pm 0.005 m/z$  of a contemporaneously analyzed Positive Control and/or the theoretical accurate mass value of the ion of interest.

### 10.3 Gas Chromatography/Mass Spectrometry

- Peaks should show good chromatographic characteristics with reasonable peak shape, width, and resolution.
- The retention time of a compound of interest should be within  $\pm 2\%$  of the retention time of a contemporaneously analyzed Positive Control.
- The signal intensity (integrated area) for a peak of interest should be  $\geq 10$  times the signal intensity for a peak of the same compound present in an injection immediately prior, as well as in the associated Negative Control(s).
- The mass spectrum of the peak of interest should compare favorably with that of a contemporaneously analyzed Positive Control. See *Guidelines for Comparison of Mass Spectra* (GenChem 33) for further guidance.

## 10.4 Other Tests

All comparison tests (e.g., chemical color tests) should compare favorably to the corresponding Positive Control.

## 11 Limitations

- The available sample size may limit or preclude some analytical techniques from being performed.
- Isomeric forms of a compound may not be differentiated by the techniques in this SOP. If relevant isomeric forms of a compound are not differentiated, this will be clearly stated in the *Laboratory Report*.

## 12 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.

## 13 References

Moffat AC, Osselton MD, Widdop B, Watts J. *Clarke's Analysis of Drugs and Poisons*, 4th ed., Pharmaceutical Press: 2011

Drug Enforcement Administration, Office of Forensic Sciences, *Analysis of Drugs Manual*, Revision 4, September 2019

*The Merck Index Online*, Royal Society of Chemistry

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

Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), *SWGDRUG Monographs*, [www.swgdrug.org](http://www.swgdrug.org)

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*, 2<sup>nd</sup> Edition, 2016

*Analysis of Tablets and Capsules*; FBI Laboratory Chemistry Unit – General Chemistry SOP

*Sampling Guidelines for Bulk Materials and Multi-Unit Populations*; FBI Laboratory Chemistry Unit – General Chemistry SOP

*Guidelines for Comparison of Mass Spectra*; FBI Laboratory Chemistry Unit – General Chemistry SOP

*Chemistry Unit Procedures for Estimating Measurement Uncertainty*; FBI Laboratory Chemistry Unit – Quality Assurance and Operations Manual

*Chemistry Unit Validation of Analytical Procedures*; FBI Laboratory Chemistry Unit – Quality Assurance and Operations Manual

*FBI Laboratory Safety Manual*



Rev. #	Issue Date	History
2	05/26/11	Added DART to section 3. Updated section 4. Revised sections 5.2 a and b. Added new instructions for the preparation of chlorinated derivatives of MAAQ, section 5.2c. Revised section 8c and 8g. Revised section 10.2 DART/TOF-MS instrument parameters to give more detail. Revised section 11.1.2f. Revised section 13e. Corrected reference in section 15.
3	01/15/20	Removed "Subunit" throughout. Removed previous section 1 (Introduction), section 3 (Principle), and section 7 (Calibration), and renumbered sections 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. Edited new sections 3.1 and 3.2 to add detail. Minor edits to sections 4 through 4.3 for clarity and to remove unnecessary detail. Section 5 edited to add more detail and to incorporate 'non-statistical' sampling language. Revised entirety of section 6 to include more detail and added DART/TOFMS as a routine technique. Minor edits made to section 8 for clarity. Changed the format of section 9 and added more detail to include flexibility for instrumental conditions. Revised section 10 for format and to include criteria for DART/TOFMS. Reformatted section 13 and updated content.

**Approval**

Redacted - Signatures on File

Chemistry Unit Chief:

Date: 01/14/2020

General Chemistry  
Technical Leader:

Date: 01/14/2020

## **Analysis of Tablets and Capsules**

### **1 Scope**

This procedure is used for the analysis of prescription, over-the-counter, and illicit tablets, capsules, pills, and similar preparations. These preparations usually consist of several components (e.g., coating, binders, active ingredient), however this procedure is designed to aid in the qualitative identification of the active ingredient(s). While the below procedure describes many techniques, the identification of a drug relies upon positive results from two orthogonal techniques with at least one of the techniques providing structural elucidation information.

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

### **2 Equipment/Materials/Reagents**

- Common laboratory glassware and equipment
- Analytical balance
- Digital microscope
- Stereo microscope
- Ultraviolet light source
- Acetaldehyde
- Acetonitrile
- Ammonium hydroxide
- Chloroform
- Deionized water
- Diethyl ether
- Formaldehyde (40%)
- Hydrochloric acid
- Methanol (MeOH)
- Nitric acid
- Sodium bicarbonate
- Sodium carbonate
- Sodium nitroprusside (aka sodium nitroferricyanide)
- Sodium sulfate (anhydrous)
- Sulfuric acid
- Evaporator

- Fourier Transform Infrared (FTIR) spectrophotometer with Attenuated Total Reflectance (ATR) or microscope attachment
- Polyethylene glycol (PEG, 550 average molecular weight)
- 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)
- Gas chromatograph/mass spectrometer (GC/MS) equipped with chemical ionization and a 30 meter DB-5 column (or equivalent)

### **3 Standards and Controls**

#### **3.1 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.

#### **3.2 Positive Control**

Prepared by making a 1 mg/mL (as base) stock standard solution of the drug into a suitable solvent. A working standard solution of 0.1 mg/mL is typically used with GC/MS by diluting the stock standard 1:10. These solutions will be stored in a freezer or refrigerator. Other concentrations may be prepared and used as needed.

### **4 Preparation of Reagents**

#### **4.1 Marquis Reagent**

Prepared by adding 8-10 drops of 40% formaldehyde to 10 mL of concentrated sulfuric acid. This solution is stored at room temperature in an amber glass bottle. Discard the solution when it begins to discolor.

#### **4.2 Sodium Nitroprusside Reagent**

- Reagent A- Prepared by dissolving 1.1 grams of sodium nitroprusside (aka sodium nitroferricyanide) into 100 mL of deionized water and 4 mL of acetaldehyde. This solution is stored in an amber glass bottle in a refrigerator.

- Reagent B- Prepared fresh by dissolving 2 grams of sodium carbonate in 100 mL of deionized water. A small amount of solid sodium carbonate (or sodium bicarbonate) can be used in lieu of the aqueous solution.

### 4.3 Hydrochloric Acid [0.1 N (aq)]

Always add acid to water. Prepared by dissolving 1.7 mL concentrated hydrochloric acid into 183.4 mL of deionized water. This solution is stored at room temperature in a glass bottle.

## 5 Sampling

Sampling is performed according to the *Sampling Guidelines for Bulk Materials and Multi-Unit Populations*– General Chemistry SOP manual.

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

## 6 Procedure

- a. Record the total tablet count. If applicable, use a traceable analytical balance to record the weight for each item.
- b. Perform a visual examination of each item and record relevant characteristics (e.g., size, shape, color, imprints, logos, score marks). Microscopy may be used as deemed necessary.
- c. Search any markings/imprints against a resource such as the *Drugs.com Pill Identifier*, *The DEA Logo Index for Tablets and Capsules*, or similar and record relevant information. Active ingredient and dosage information can be used in combination with the total weight of the item to determine the amount of an item necessary to yield a desired amount of active ingredient for extraction and solution preparation. Counterfeit items may contain different amounts, types, and/or additional active ingredients.
- d. Color tests may be performed. A spot plate should be used and all samples (e.g., controls, items) will be added to the reagent to ensure that the spot plate well is free of contamination. Color tests not described below may be prepared and used following similar practices. Include a copy of the reference relied upon for the color test. Examples of resources include *Clarke's Analysis of Drugs and Poisons*, SWGDRUG Drug Monographs, and the *DEA Analysis of Drugs Manual*.

#### Marquis Test:

- Add 2-3 drops of Marquis Reagent to the required number of spot plate wells. Add samples to separate wells and observe and record any changes. One well will remain unaltered during the exam to demonstrate the Marquis Reagent does not change color spontaneously. A purple color indicates the possible presence of an opiate. A violet to black color indicates the possible presence of 3,4-methylenedioxyamphetamine (MDA) or 3,4-methylenedioxymethamphetamine (MDMA). An orange color indicates the possible presence of an amphetamine compound.

#### Nitroprusside Test:

- Add 2-3 drops of Sodium Nitroprusside Reagent A to the required number of spot plate wells. Add samples to separate wells and observe and record any changes. Next, add 2-3 drops of Sodium Nitroprusside Reagent B (or a small amount of solid sodium carbonate or sodium bicarbonate) to each well and observe and record any changes. One well will only have Reagents A and B added to it to demonstrate that a color change does not occur. A blue or violet color upon addition of Reagent B indicates the possible presence of an amphetamine compound.

#### Concentrated Nitric Acid Test:

- Add 2-3 drops of concentrated nitric acid to the required number of spot plate wells. Add samples to separate wells and observe and record any changes. One well will remain unaltered during the exam to demonstrate the nitric acid does not change color spontaneously. An orange color that fumes indicates the possible presence of acetaminophen. An orange color that does not fume indicates the possible presence of morphine or codeine. A lime green color indicates the possible presence of guaifenesin or methocarbamol. A blue fluorescence under ultraviolet light indicates the possible presence of quinine.
- e. Analyze a representative sample of the item by FTIR-ATR. A tablet/pill may be homogenized using a mortar and pestle (if possible, homogenize approximately one-half of the item while retaining the other half intact). A capsule may be opened to remove solid contents or a syringe with needle may be used to remove liquid contents. Liquid samples may be analyzed neat and/or allowed to evaporate on the ATR cell. The FTIR microscope attachment may be used as appropriate.
- f. An appropriate amount of the item may be placed into a small test tube and extracted or dissolved in an appropriate solvent (e.g., methanol, chloroform, acetonitrile) to achieve the

desired concentration. For example, a concentration of ~1 mg/mL may be desirable for DART/TOFMS analysis, while a concentration of ~100 ug/mL is common for GC/MS analysis. If necessary, filter the solution or centrifuge and decant to remove any undissolved particulates. The Negative Control will be filtered or centrifuged as well. It may be necessary to utilize acidic or basic conditions to more efficiently extract some drugs, see Appendix A for acid/neutral and alkaline drug extraction steps.

- g. The solution from (f.) may be analyzed by color tests [see step (d.)]. A Negative Control will be analyzed along with the item extract(s).
- h. The solution from (f.) may be analyzed by DART/TOFMS in positive and/or negative ionization mode (as appropriate based on the target analyte) by sampling the solution with the closed end of a glass capillary. Analyze the Negative Control(s), the Positive Control(s) (if applicable at this point), and PEG within the same data collection file. If the Positive Control(s) is determined after the initial DART/TOFMS analysis, then analyze the Positive Control(s) and PEG within a separate data collection file. It is also acceptable to analyze an item prior to extraction/dilution. For powder samples, a glass capillary can be wetted with deionized water and then touched to the sample; collect a blank glass capillary wetted with deionized water as a Negative Control.
- i. The solution from (f.) may be analyzed by GC/MS in the electron impact (EI) mode. Also analyze the Negative Control and Positive Control(s) (if applicable at this point), and incorporate a solvent blank between each sample. If the Positive Control(s) is determined after the initial GC/MS analysis, then analyze the Positive Control(s) within a separate sequence.
- j. The solution from (f.) may be analyzed by GC/MS in the positive ion chemical ionization (PICI) or negative ion chemical ionization (NICI) mode as appropriate. Also analyze the Negative Control and a Positive Control (if applicable at this point), and incorporate a solvent blank between each sample. If the Positive Control(s) is determined after the initial GC/MS analysis, then analyze the Positive Control(s) within a separate sequence. If an amphetamine (or other drug which gives a limited EI spectrum) is suspected, and FTIR or DART/TOFMS has not been utilized (or did not provide sufficient information), then PICI will be performed. A basic extraction using sodium bicarbonate and chloroform may improve the chromatography of methamphetamine.
- k. Other analytical techniques not listed above may be used to analyze a drug as deemed necessary {e.g., X-ray Powder Diffractometry (XRD), Liquid Chromatography/Mass Spectrometry [LC/MS, with electrospray (ESI) or atmospheric-pressure chemical ionization (APCI)], Raman spectrophotometry, Ultraviolet-Visible (UV-Vis) spectroscopy, Thin-Layer Chromatography (TLC)} provided that the instrumental conditions are retained in the case

notes, the Negative Control and Positive Control samples provide the appropriate responses, and any relied upon references are retained in the case notes. These techniques are reserved for instances when the preceding steps don't provide sufficient data to identify a drug. If it is anticipated that the technique will be used routinely in the future for the drug, then the technique will be validated per the *Chemistry Unit Validation of Analytical Procedures* (CU QAOM 11).

## 7 Calculations

Following is an example calculation for preparing a 1 mg/mL extraction solution of oxycodone from a tablet (e.g., small, round, light blue tablet with "M" and "30" imprints).

$$\frac{30 \text{ mg oxycodone HCl}}{108.7 \text{ mg tablet weight}} \times \frac{315.37 \text{ mg oxycodone}}{351.83 \text{ mg oxycodone HCl}} \times 100 = 24.7 \text{ wt. \% oxycodone}$$

$$\frac{X \text{ mg tablet} \times 24.7 \text{ wt. \% oxycodone}}{2.5 \text{ mL MeOH}} = \frac{1 \text{ mg oxycodone}}{1 \text{ mL MeOH}}$$

$$X \text{ mg tablet} = \frac{1 \text{ mg oxycodone}}{1 \text{ mL MeOH}} \times \frac{2.5 \text{ mL MeOH}}{24.7 \text{ wt. \% oxycodone}}$$

$$X \text{ mg tablet} = 10.1 \text{ mg}$$

For this example, weigh and transfer 10.1 mg of the homogenized item to a labeled test tube, then add 2.5 mL MeOH to yield an oxycodone solution of ~ 1 mg/mL (assuming 100% recovery).

## 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 on the CU s:\ drive.

## 9 Instrumental Conditions

The following instrumental conditions are typically used for drug analysis. Minor modifications to the conditions may be used as needed and without authorization, provided the same conditions are used for applicable solvent blanks, Negative Control(s), questioned items, and Positive Control(s); and the Positive Control(s) provide acceptable data. The utilized conditions will be retained with the case notes. [Note- some of the parameters are unique to an individual instrument and will be different among similar instruments (e.g., tune parameters).]

### 9.1 FTIR

#### 9.1.1 Benchtop with ATR

- Scan range: 4000-400  $\text{cm}^{-1}$
- Scans: 32 (sample and background scans)
- Resolution: 4  $\text{cm}^{-1}$

#### 9.1.2 Microscope

- Scan range: 4000-650  $\text{cm}^{-1}$
- Scans: 128
- Resolution: 4  $\text{cm}^{-1}$

### 9.2 DART/TOFMS

DART Source Settings	
Polarity	Positive or Negative
Source gas	Helium
Temperature	400 °C

TOFMS Settings	
Polarity	Positive or Negative
Ion Guide RF (or equivalent)	800 V for 80-800 $m/z$ , 500 V for 50-500 $m/z$



## 9.3 Gas Chromatography/Mass Spectrometry (GC/MS)

### 9.3.1 Electron Impact (EI)

GC Settings	
Injection Mode	Split or Splitless (sample dependent; consistent across blanks, controls, samples)
Injection Volume	1 uL
Inlet Temperature	250 °C
Oven Program	60 °C for 2 min, 35 °C /min to 260 °C for 15 min [some analytes require longer final times and/or higher final temperatures (e.g., steroids, heavier analytes)]
MS Settings	
Polarity	Positive
Scan Range	43 to 400 $m/z$ (increase final $m/z$ for heavier target analytes to 50 $m/z$ greater than molecular ion)

### 9.3.2 Chemical Ionization (CI)

GC Settings	
Injection Mode	Split or Splitless (sample dependent; consistent across blanks, controls, samples)
Injection Volume	1 uL
Inlet Temperature	250 °C
Oven Program	60 °C for 2 min, 35 °C /min to 260 °C for 15 min [some analytes require longer final times and/or higher final temperatures (e.g., steroids, heavier analytes)]
MS Settings	
Polarity	Positive or Negative
Reagent Gas	Methane
Scan Range	100 to 500 $m/z$ (increase final $m/z$ for heavier target analytes to 50 $m/z$ greater than molecular ion)

## 10 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of data produced in this procedure.

## 10.1 FTIR

- The FTIR spectrum for the analyte of interest should compare favorably to the corresponding Positive Control (or library) spectrum.
- If FTIR is the only instrumental technique used, a Positive Control of the analyte of interest must be contemporaneously analyzed on the same instrument.

## 10.2 DART/TOFMS

Peaks of interest should be within  $\pm 0.005 m/z$  of a contemporaneously analyzed Positive Control and/or the theoretical accurate mass value of the ion of interest.

## 10.3 Gas Chromatography/Mass Spectrometry

- Peaks should show good chromatographic characteristics with reasonable peak shape, width, and resolution.
- The retention time of a compound of interest should be within  $\pm 2\%$  of the retention time of a contemporaneously analyzed Positive Control.
- The signal intensity (integrated area) for a peak of interest should be  $\geq 10$  times the signal intensity for a peak of the same compound present in an injection immediately prior, as well as in the associated Negative Control(s).
- The mass spectrum of the peak of interest should compare favorably with that of a contemporaneously analyzed Positive Control. See *Guidelines for Comparison of Mass Spectra* (GenChem 33) for further guidance.

## 10.4 Other Tests

All comparison tests (e.g., chemical color tests) should compare favorably to the corresponding Positive Control.

## 11 Limitations

- The available sample size may limit or preclude some analytical techniques from being performed.
- Some imprints/markings/logos may not be listed in a resource.
- Isomeric forms of a compound may not be differentiated by the techniques in this SOP. If relevant isomeric forms of a compound are not differentiated, this will be clearly stated in the *Laboratory Report*.

## 12 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.

## 13 References

Moffat AC, Osselton MD, Widdop B, Watts J. *Clarke's Analysis of Drugs and Poisons*, 4th ed., Pharmaceutical Press: 2011

Drug Enforcement Administration, Office of Forensic Sciences, *Analysis of Drugs Manual*, Revision 4, September 2019

*The Merck Index Online*, Royal Society of Chemistry

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

Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), *SWGDRUG Monographs*, [www.swgdrug.org](http://www.swgdrug.org)

Drug Enforcement Administration, Office of Forensic Sciences, *Logo Index for Tablets and Capsules*

Drugs.com *Pill Identifier*, [www.drugs.com/pill\\_identification.html](http://www.drugs.com/pill_identification.html)

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

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*Drug Analysis*; FBI Laboratory Chemistry Unit – General Chemistry SOP

*Sampling Guidelines for Bulk Materials and Multi-Unit Populations*– General Chemistry SOP manual

*Guidelines for Comparison of Mass Spectra*; FBI Laboratory Chemistry Unit – General Chemistry SOP

*Chemistry Unit Procedures for Estimating Measurement Uncertainty*; FBI Laboratory Chemistry Unit – Quality Assurance and Operations Manual

*Chemistry Unit Validation of Analytical Procedures*; FBI Laboratory Chemistry Unit – Quality Assurance and Operations Manual

*FBI Laboratory Safety Manual*

Rev. #	Issue Date	History
2	05/26/11	Added DART to section 3. Updated section 4. Revised sections 5.2 a and b. Added new instructions for the preparation of chlorinated derivatives of MAAQ, section 5.2c. Revised section 8c and 8g. Revised section 10.2 DART/TOF-MS instrument parameters to give more detail. Revised section 11.1.2f. Revised section 13e. Corrected reference in section 15.
3	01/15/20	Removed “Subunit” throughout. Removed previous section 1 (Introduction), section 3 (Principle), and section 7 (Calibration), and renumbered sections 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. Edited new sections 3.1 and 3.2 to add detail. Minor edits to sections 4 through 4.4 for clarity and to remove unnecessary detail. Section 5 edited to add more detail and to incorporate ‘non-statistical’ sampling language. Revised entirety of section 6 to include more detail and added DART/TOFMS as a routine technique. Added content to section 7. Minor edits made to section 8 for clarity. Changed the format of section 9 and added more detail to include flexibility for instrumental conditions. Revised section 10 for format and to include criteria for DART/TOFMS. Minor edit made to second bullet in section 11. Reformatted section 13 and updated content. Added Appendix A.

### Approval

Redacted - Signatures on File

Chemistry Unit Chief:

Date: 01/14/2020

General Chemistry  
 Technical Leader:

Date: 01/14/2020

## **Appendix A: Acid/Neutral and Alkaline Drug Extractions**

### **Acid/Neutral Drug Extraction-**

Mix several milligrams of a homogenized tablet with several milliliters of deionized water in a test tube. Acidify the solution with 0.1 N hydrochloric acid until a pH of ~ 2 is achieved (check with pH paper). Add several milliliters of chloroform and rotate the mixture for approximately 10 minutes. Isolate the bottom chloroform layer and filter through pre-rinsed anhydrous sodium sulfate. Collect the chloroform layer into a labeled test tube and concentrate the solution under N<sub>2</sub> (g) flow at ~60 °C.

### **Alkaline Drug Extraction-**

Mix several milligrams of a homogenized tablet with several milliliters of deionized water in a test tube. Add sodium bicarbonate until a pH of ~10 is achieved (check with pH paper). Add several milliliters of chloroform and rotate the mixture for approximately 10 minutes. Isolate the bottom chloroform layer and filter through pre-rinsed anhydrous sodium sulfate. Collect the chloroform layer into a labeled test tube and concentrate the solution under N<sub>2</sub> (g) flow at ~60 °C.

## Analysis of Delta-9-tetrahydrocannabinol

### 1 Scope

This procedure is used for the analysis of items suspected of containing delta-9-tetrahydrocannabinol ( $\Delta^9$ -THC or THC, the major psychoactive compound in marijuana).

This procedure applies to Chemistry Unit (CU) personnel that are qualified to examine 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 ( $\Delta^9$ -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

- $\Delta^9$ -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

Sampling of multiple items is performed according to the *Sampling Guidelines for Bulk Materials and Multi-Unit Populations*– General Chemistry SOP manual.



## 6 Procedure

- 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  $\Delta^9$ -THC.
- c. Weigh (record the weight) and transfer ~ 10 mg of the item to a labeled test tube. An item may have packaging with  $\Delta^9$ -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.
- d. Isolate the extract solutions by transferring the solutions to new labeled test tubes. If necessary, concentrate the extract(s) under N<sub>2</sub> (g) flow at 60 °C. The associated Negative Control extract will be concentrated in the same manner as the extract solutions.
- e. 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  $\Delta^9$ -THC Positive Control, and PEG within the same data collection file.
- f. 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  $\geq$  1 cm from bottom of TLC plate). Spot 5 to 10 uL of the  $\Delta^9$ -THC Positive Control and the Negative Control(s) extract(s) 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.
- g. Analyze the extracts by GC/MS using electron impact (EI) ionization mode. Also analyze the Negative Control(s) and the  $\Delta^9$ -THC Positive Control, and incorporate a solvent blank between each sample.

## 7 Calculations

### 7.1 Thin-layer Chromatography (TLC)

- Distances traveled are measured from the origin
- Retardation factor ( $R_f$ ) =  $\frac{\text{distance traveled by center of spot}}{\text{distance traveled by solvent}}$

## 8 Measurement Uncertainty

When quantitative results (i.e., weight) are included in a Laboratory Report, the estimated measurement uncertainty will be reported according to the *Chemistry Unit Procedures for Estimating Measurement Uncertainty*.

## 9 Instrumental Conditions

The following instrumental conditions are typically used for this analysis. Minor modifications to the conditions may be used as needed and without authorization, provided the same conditions are used for all 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. [Note- some of the parameters are unique to an individual instrument and will be different among similar instruments (e.g., column specifics, tune parameters).]

### 9.1 Time-of-Flight Mass Spectrometry with Direct Analysis in Real Time (DART/TOFMS)

DART Source Settings	
Polarity	Positive
Source gas	Helium
Temperature	400 °C

TOFMS Settings	
Polarity	Positive
Ion Guide RF (or equivalent)	800 V for 80-800 $m/z$ , 500 V for 50-500 $m/z$

## 9.2 Gas Chromatography/Mass Spectrometry (GC/MS)

### 9.2.1 Electron Impact (EI)

GC Settings	
Injection Mode	Split or Splitless (sample dependent; consistent across blanks, controls, samples)
Injection Volume	1 uL
Inlet Temperature	250 °C
Oven Program	60 °C for 2 min, 35 °C /min to 260 °C for 15 min
MS Settings	
Polarity	Positive
Scan Range	43 to 400 $m/z$

## 10 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of data produced in this procedure.

### 10.1 Thin Layer Chromatography

The  $R_f$  value(s) of the spot(s) from the questioned item(s) should be within  $\pm 10\%$  of the  $R_f$  value(s) of the spot(s) for the corresponding Positive Control(s).

### 10.2 DART/TOFMS

Peaks of interest should be within  $\pm 0.005 m/z$  of a contemporaneously analyzed Positive Control and/or the theoretical accurate mass value of the ion of interest.

### 10.3 Gas Chromatography/Mass Spectrometry

- Peaks should show good chromatographic characteristics with reasonable peak shape, width, and resolution.
- The retention time of a compound of interest should be within  $\pm 2\%$  of the retention time of a contemporaneously analyzed Positive Control.
- The signal intensity (integrated area) for a peak of interest should be  $\geq 10$  times the signal intensity for a peak of the same compound present in an injection immediately prior, as well as in the associated Negative Control(s).

- The mass spectrum of the peak of interest should compare favorably with that of a contemporaneously analyzed Positive Control. See *Guidelines for Comparison of Mass Spectra* (GenChem 33) for further guidance.

## 11 Limitations

- The available sample size may limit or preclude some analytical techniques from being performed.
- Older items may have minimal amounts of  $\Delta^9$ -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.

## 12 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.

## 13 References

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

Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), *SWGDRUG Monographs*, [www.swgdrug.org](http://www.swgdrug.org)

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

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European Network of Forensic Science Institutes (ENFSI), *Guidelines on Sampling of Illicit Drugs for Qualitative Analysis*, 2<sup>nd</sup> 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

*Guidelines for Comparison of Mass Spectra*; FBI Laboratory Chemistry Unit – General Chemistry SOP

*Chemistry Unit Procedures for Estimating Measurement Uncertainty*; FBI Laboratory Chemistry Unit – Quality Assurance and Operations Manual

*FBI Laboratory Safety Manual*

Rev. #	Issue Date	History
3	03/08/12	Added time-of-flight mass spectrometry to section 3. Minor wording changes to sections 5a, 6.2, and 6.3. Added digital microscope and DART-TOF/MS to section 4. Split step 9a into two steps 9a-b. Slight wording changes to sections 9i and 9j. Added DART-TOF/MS as an alternative method to TLC or color test section 9k. Added the use of controls to section 9l. Added DART-TOF instrument conditions to section 10. Added a statement to section 12.1.2f regarding the ions used for mass spectrometry decision criteria. Added Table 1 to section 12.1.2. Added LOD to section 14.
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).

**Approval**

Redacted - Signatures on File

Chemistry Unit Chief:

Date: 01/14/2020

General Chemistry  
Technical Leader:

Date: 01/14/2020

## **Sampling Guidelines for Bulk Materials and Multi-Unit Populations**

### **1 Scope**

This procedure describes statistical and non-statistical sampling approaches for General Chemistry items consisting of bulk material or multi-unit populations. Due to the nature of the cases and items that General Chemistry analyzes, no one approach is sufficient. For all item types, additional samples may be examined as deemed necessary. For example, a product tampering case may necessitate the analysis of all units within a population.

This procedure applies to Chemistry Unit (CU) personnel that are qualified to examine General Chemistry evidence.

### **2 Equipment/Materials/Reagents**

Refer to the appropriate SOP for the complete list of equipment, materials, and reagents.

### **3 Standards and Controls**

Refer to the appropriate SOP.

### **4 Sampling**

See Section 5.

### **5 Procedure**

#### **5.1 Bulk Materials**

##### **5.1.1 Homogeneous**

A single, representative sample may be selected for analysis from a bulk material that is visually homogeneous (e.g., single layer liquids, solids with evenly distributed components).

### 5.1.2 Heterogeneous

A single, representative sample may be selected for analysis from a bulk material that is visually heterogeneous. The *Laboratory Report* shall clearly limit the results of examinations to the sample that was examined.

If the results of examinations are to be applied to the entirety of the item, then the material will be homogenized prior to analysis. This may be accomplished by sampling multiple, representative areas of the item and homogenizing with a mortar and pestle. Alternatively, the sample may be broken down into multiple, homogeneous samples prior to analysis (e.g., particle picking). The details of the sampling approach will be recorded in the case notes and the *Laboratory Report* will clearly communicate any limitations.

## 5.2 Multi-Unit Populations

An item can be treated as a multi-unit population if it consists of 2 or more units that have similar physical properties (e.g., color, shape, size, logos, markings, imprints).

### 5.2.1 Non-Statistical Sampling

One or more units may be randomly selected for analysis. When non-statistical sampling is utilized, the *Laboratory Report* shall clearly limit the results of examinations to the units that were examined.

### 5.2.2 Statistical Sampling

As appropriate, the contributor will be contacted to determine if an inference is necessary, or if non-statistical sampling is sufficient. The details of this conversation will be recorded on the applicable *Communication Log*. If an inference is to be made about the population, then statistical sampling will be performed. See Appendix A to determine the number of units to randomly select for analysis (without replacement). The *Hypergeometric Table* utilizes a 95% confidence interval for an inference about 90% of the population. Adjustments to the table will be necessary if the confidence interval and/or population % are changed. Additionally, Appendix A assumes that all results are consistent. If inconsistent results are encountered, additional units may need to be analyzed and/or adjustments to the inference values may be required (see Appendix B).



## 6 Calculations

Following is an example inference made utilizing statistical sampling of a population. See Appendices A and B for further details.

Item 1 consisted of 263 small, round, light blue tablets with “M” and “30” imprints (indicative of 30 mg oxycodone hydrochloride tablets). Per the *Hypergeometric Table* (Appendix A), 27 tablets were randomly selected and sub-itemized (Items 1-1 through 1-27). Oxycodone was identified within each of the Item 1-1 through 1-27 tablets.

A combination of the results of examinations and the sampling plan utilized allows for the inference that  $\geq 236$  of the 263 submitted tablets would be expected to contain oxycodone. This inference is made at a 95% confidence level.

## 7 Measurement Uncertainty

Not applicable.

## 8 Limitations

When non-statistical sampling is used, the *Laboratory Report* will clearly state that the results are limited to the sample(s) or portion(s) of sample(s) that was analyzed.

If inconsistent results are obtained during the analysis of the samples selected under section 5.2.2, then the proportion of the population will be adjusted to maintain a 95% confidence level for an inference about the population. An excel spreadsheet is maintained on the CU share drive to calculate the new proportion value. An example calculation can be found in Appendix B.

## 9 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.

## 10 References

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

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*, 2<sup>nd</sup> Edition, 2016

Frank RS, Hinkley SW, Hoffman CG. Representative sampling of drug seizures in multiple containers, *J Forensic Sci* 1991; 36(2): 350-357

*FBI Laboratory Safety Manual*

Rev. #	Issue Date	History
3	07/03/13	Deleted section 8.2 Sampling for Quantitative Analysis. Updated references. Added titles to Appendices.
4	01/15/20	Revised Title. Removed 'subunit' throughout. Removed previous section 1 (Introduction), section 3 (Principle), and section 6 (Calibration), and renumbered sections accordingly. Edited new section 1 for clarity and to include personnel. Reformatted section 5 and revised approach to include broader array of sample types (was primarily specific to pills, capsules, tablets, etc.). Renamed section 7 (was 'Uncertainty of Measurement'). Minor edits made to section 8 for clarity. Reformatted section 10 and updated content. Deleted last sentence from paragraph in Appendix A and added "(no inferences)" to second row in table. Revised the example calculation in Appendix B.

**Approval**

Redacted - Signatures on File

Chemistry Unit Chief:

Date: 01/14/2020

General Chemistry  
Technical Leader:

Date: 01/14/2020

## Appendix A: *Hypergeometric Table*

The hypergeometric table listed below shows the minimum number of samples that need to be analyzed (and yield consistent results) to obtain a 95% confidence level that at least 90% of the population contains a given substance.

Total Number of Units	Number of Units to be Sampled
1-10	All (no inferences)
11-13	10
14	11
15-16	12
17	13
18	14
19-24	15
25-26	16
27	17
28-35	18
36-37	19
38-46	20
47-48	21
49-58	22
59-77	23
78-88	24
89-118	25
119-178	26
179-298	27
299-1600	28
more than 1600	29

## Appendix B: Example for Estimating Proportion of Population

Following is an example of how to estimate the proportion of the population that contains a substance for the situation where 1 or more randomly selected samples provided negative or inconsistent results:

A submission to the laboratory consisted of 125 physically similar tablets (no manufacturer's logos). Per the table in Appendix A, 26 samples were randomly selected and analyzed. MDMA was identified in 25 of the samples, while no controlled substances were identified within 1 of the samples. The "NUMBER OF SUCCESSES IN THE SAMPLE", "SAMPLE SIZE", and "POPULATION SIZE" cells were entered in the below table (25, 26, 125, respectively). The entry in the "NUMBER OF SUCCESSES IN THE POPULATION" cell was then varied until the "% CONFIDENCE LEVEL" cell became just  $\geq 95\%$ . At that point, take note of the value in the "% POPULATION THAT CONTAINS SUBSTANCE" cell. This is the new value that would replace the value "90%" for the situation where all samples selected were consistent when analyzed. For this example, the report would read to the effect "at a 95% confidence level, it is estimated that at least 106 tablets contained MDMA".

NUMBER OF SUCCESSES IN THE SAMPLE (X)	25
SAMPLE SIZE (n)	26
INFERRED NUMBER OF SUCCESSES IN THE POPULATION*** (R)	106
POPULATION SIZE (N)	125
PROB. of X successes for above n, R, N	0.048421
*** VARY THIS VALUE SUCH THAT % CONF. LEVEL $\geq 95\%$	
% CONFIDENCE LEVEL	95.2
INFERRED % POPULATION THAT CONTAINS SUBSTANCE	84.8

## **Synthetic Human Growth Hormone (Somatropin) Analysis by LC/MS(ESI)**

### **1 Introduction**

Human growth hormone (hGH) is a protein produced by the pituitary gland that regulates growth and metabolism. It is composed of 191 amino acids and has an approximate molecular weight of 22,125 Daltons. Many legitimate medical uses of hGH exist. However, illegitimate uses such as abuse by athletes to increase muscular strength and growth, often in combination with steroids, are also common.

Many commercial hGH preparations contain Somatropin, which is a synthetic form of hGH that consists of the same sequence of 191 amino acids as naturally occurring hGH. These preparations are typically provided in a lyophilized powdered form along with a fixed volume of diluent to prepare a specific concentration. A variety of substances are used as diluents. Examples of diluents include bacteriostatic water and saline.

Since proteins can carry multiple charges in solution, multiple charge states of synthetic hGH (Somatropin) can be examined using liquid chromatography/electrospray ionization mass spectrometry (LC/MS(ESI)).

### **2 Scope**

This procedure allows for the verification of labeling of pharmaceutical preparations of synthetic human growth hormone (Somatropin).

### **3 Principle**

Samples are diluted to an appropriate concentration before analysis. Separation is performed using a Zorbax 300SB-C<sub>3</sub> column (or equivalent). Detection is by LC/MS(ESI).

### **4 Specimens**

This procedure is used to analyze labeled, pharmaceutical preparations of synthetic human growth hormone (Somatropin).

## 5 Equipment/Materials/Reagents

- a. Liquid Chromatograph/Mass Spectrometer (ion trap) operating in the electrospray ionization mode and equipped with a Zorbax 300SB-C<sub>3</sub> (150 mm x 2.1 mm x 5 µm particle column) (or equivalent)
- b. Balance accurate to at least  $\pm 0.2$  mg
- c. Adjustable pipetters (0.005 mL to 1 mL) with appropriate tips
- d. Volumetric flasks
- e. Routine laboratory supplies, including disposable glass pipets, spatulas, autosampler vials, test tube racks, graduated cylinders, etc.
- f. Acetonitrile (Optima grade)
- g. Water (Optima grade)
- h. Trifluoroacetic Acid (TFA)
- i. Formic Acid
- j. Mobile Phase A consisting of 0.05 % Formic Acid and 0.01 % TFA in H<sub>2</sub>O (pH ~ 2.5). Add 125 µL Formic Acid and 25 µL TFA to an appropriate, volumetric glass container. Bring to 250 mL mark with deionized H<sub>2</sub>O and mix well. Verify that the pH is ~ 2.5.
- k. Mobile Phase B consisting of 0.05 % Formic Acid and 0.01 % TFA in CH<sub>3</sub>CN (pH ~ 5). Add 125 µL Formic Acid and 25 µL TFA to an appropriate, volumetric glass container. Bring to 250 mL mark with CH<sub>3</sub>CN and mix well. Verify that the pH is ~ 5.
- l. ProMass for Xcalibur (Automated ESI Deconvolution and Web-based Reporting software for Finnigan Xcalibur) (or equivalent)

## 6 Standards and Controls

- a. Synthetic hGH (Somatropin) Injectable Solutions (e.g., 800 ug/mL GENOTROPIN MINIQUICK Growth Hormone Delivery Device, Pfizer):  
Obtained from Pfizer or an equivalent pharmaceutical supplier. Stability and storage determined by manufacturer.

- b. Somatropin Positive Control (200 ug/mL):  
Prepare the Synthetic hGH (Somatropin) Injectable Solution (see above) as directed by the manufacturer. Dilute in deionized water to achieve a concentration of 200 ug/mL. A Somatropin Positive Control will be analyzed with every hGH assay.
- c. Negative Control:  
Bacteriostatic water, saline, or other appropriate diluent is the Negative Control in this procedure. A Negative Control will be analyzed with every hGH assay.

## 7 Calibration

Not applicable.

## 8 Sampling

Sampling is performed according to the *Sampling Plan for Controlled Substances, Tablets, Capsules, and Pharmaceuticals*- General Chemistry Subunit SOP Manual.

## 9 Procedure

- a. Dilute specimens with an appropriate amount of deionized water or a diluent if submitted with samples.
- b. Verify that the LC/MS is in proper working condition by analyzing the Somatropin Positive Control (200 ug/mL) using the instrumental operating conditions in Section 10 of this procedure. The results can be compared to previous chromatograms and mass spectra located in the validation binder for this procedure.
- c. Transfer samples and controls to autosampler vials and analyze 5 µL by LC/MS(ESI).
- d. Process applicable mass spectra using ProMass for Xcalibur ESI deconvolution software.
  - 1. Export the desired mass spectrum from the Xcalibur Qual Browser (right click, Export- Clipboard- Exact Mass).
  - 2. Open ProMass for Xcalibur and click on the “Build Params” icon.



3. Load the "hGH.PARAMS" file.
4. Click on the clipboard icon, which will run the deconvolution program. A report containing the Base Peak Mass, Intensity, and Spectral Quality will be generated.

## 10 Instrumental Conditions

Following are the instrumental conditions to be used in this procedure:

### 10.1 Liquid Chromatography Parameters

Mobile Phase Compositions	Flow Parameters			Column Parameters	
A: 0.05% Formic Acid, 0.01% TFA in H <sub>2</sub> O (pH ~ 2.5)	Total Flow: 0.25 mL/min			Type	300 SB-C <sub>3</sub>
	Time (min)	%A	%B		
	0.1	90	10	Dimensions	15 cm x 2.1 mm
	2	90	10		
B: 0.05% Formic Acid, 0.01% TFA in CH <sub>3</sub> CN (pH ~ 5)	21	28	72	Particle Size	5 µm
	22	90	10		
	28	90	10	Temperature	30 °C
	Total Run Time: 28 min				

### 10.2 Mass Spectrometer Parameters

Ionization Mode	Electrospray (+)	All source parameters are set through the instrument tuning process. See the Instrument Operations and Support Subunit SOP Manual for details.
Scan Mode	Full Scan	
Scan Range	1000 – 2000 AMU	

## 11 Decision Criteria

The following criteria are used as guides in determining the acceptability of the data produced in this assay. In general, a comparison of the chromatography and mass spectrometry for the analyte peak of interest with data from a contemporaneously analyzed reference standard should meet the following criteria.

## 11.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. Additionally, the following two criteria should be met.

### 11.1.1 Retention Time

The retention time of the peak should be within  $\pm 5\%$  of the retention time obtained from injection of the Somatropin Positive Control.

### 11.1.2 Signal-to-Noise

To justify the existence of a peak, its signal to noise ratio should exceed 3. Further, the signal for the peak from the sample of interest should be at least 10 fold greater than that for any observed peak at a similar retention time in a Negative Control or blank sample injected just prior to that sample.

## 11.2 Mass Spectrometry

The mass spectra (ESI and Deconvoluted) of the analyte of interest should compare favorably to that of the appropriate reference standard within a reasonable degree of scientific certainty. See the *Guidelines for Comparison of Mass Spectra* standard operating procedure (Tox 104) for further guidance. Table 2 shows the ions that should be observed for synthetic hGH (Somatropin).

Table 1: Synthetic hGH (Somatropin) Ions

Ions Observed ( $m/z$ )	Adduct
1844-1845	[hGH + 12H] <sup>12+</sup>
1702-1703	[hGH + 13H] <sup>13+</sup>
1581-1582	[hGH + 14H] <sup>14+</sup>
1475-1476	[hGH + 15H] <sup>15+</sup>
1383-1384	[hGH + 16H] <sup>16+</sup>
1302-1303	[hGH + 17H] <sup>17+</sup>
1230-1231	[hGH + 18H] <sup>18+</sup>

## 12 Calculations

Not applicable.

### 13 Uncertainty of Measurement

Not applicable.

### 14 Limitations

- a. Limit of Detection:  $\leq 25$  ug/mL
- b.. Interferences: None known

### 15 Safety

Take standard precautions for the handling of chemicals, potentially infectious materials, and hypodermic needles. See the *FBI Laboratory Safety Manual* for guidance.

### 16 References

Wisniewski, Eric S., D.K. Rees, E.C. Chege, "Proteolytic-Based Method for the Identification of Human Growth Hormone", *Journal of Forensic Science* 54:122-127 (2009).

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Spolaore, Barbara, P. Polverino, M. Zambonin, A. Fontana, "Limited Proteolysis of Human Growth Hormone at Low pH: Isolation, Characterization, and Complementation of the Two Biologically Relevant Fragments 1-44 and 45-191", *Biochemistry* 43:6576-6586 (2004).

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*Guidelines for Comparison of Mass Spectra*; FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

*Performance Monitoring Protocol (QA/QC) for the Thermo LTQ LC/MS (ESI)*; FBI Laboratory Chemistry Unit - Instrument Operation and Support Subunit SOP Manual.

*Sampling Plan for Controlled Substances, Tablets, Capsules, and Pharmaceuticals*; FBI Laboratory Chemistry Unit – General Chemistry Subunit SOP Manual.

*FBI Laboratory Safety Manual*.

Rev. #	Issue Date	History
0	10/16/09	Original document.

**Approval**

Redacted - Signatures on File

## General Approach to Report Writing in General Chemistry

### 1 Scope

Reports issued by General Chemistry examiners summarize analytical findings. Due to the wide variety of requests and evidence received, this standard operating procedure is only a general guideline for report writing. It will not always be possible to write a report using only the examples provided here. It is acceptable to use other wording as long as the results of the examinations are accurately communicated, a summary of the methodology used to reach the results is included, any known limitations are addressed, and the wording is approved by a second examiner who is qualified in the discipline during the technical review process. Additionally, any wording must comply with the *FBI Approved Standards for Scientific Testimony and Report Language for the General Chemistry Discipline* (GenChem ASSTR) and the *Department of Justice Uniform Language for Testimony and Reports for General Forensic Chemistry and Seized Drug Examinations* document (GenChem ULTR) documents.

This procedure applies to Chemistry Unit (CU) personnel that are qualified to author *Laboratory Reports* in General Chemistry.

### 2 Equipment/Materials/Reagents

Not applicable.

### 3 Standards and Controls

Not applicable.

### 4 Sampling

Not applicable.

## 5 Procedure

- a. Prepare and format the *Laboratory Report* in accordance with requirements set forth in the *FBI Laboratory Operations Manual*. Prepare a **Results of Examinations** section, an **Interpretations/Limitations** section when applicable, and a **Remarks** section.
- b. The **Results of Examinations** section will be used to communicate the results of the General Chemistry examinations and a summary of the methodology used, and will include the requirements set forth in the *FBI Laboratory Operations Manual*. This section may also include a description of the items received or other information to assist in communicating the results. The below list contains guidance for the **Results of Examinations** section for General Chemistry reports. Examples of appropriate wording for the **Results of Examinations** section are included in Appendix A.
  - Include the units of quantitative results. When using an abbreviation for the units for the first time in a report, define the abbreviation for clarity.
  - Include the estimated measurement uncertainty value and the confidence level when reporting a quantitative value that was measured in the laboratory.
  - Report measurement uncertainty values to no more than two significant figures. When measurement uncertainty values are rounded, always round up.
  - Report the measurand to the same level of significance as the measurement uncertainty. Always truncate the measurand (e.g.,  $125.7 \pm 25.2$  will be reported as  $125 \pm 26$ ).
  - Tables may be used to summarize results as long as all applicable elements above are included in the **Results of Examinations** section.
- c. The **Interpretations/Limitations** section will be used to communicate any known limitations of the results, or limitations of the testing based on the evidence received. This section will also include any interpretations that may aid the reader in understanding the significance of the **Results of Examinations**. The below list contains guidance for the **Interpretations/Limitations** section for General Chemistry reports. Examples of appropriate wording for the **Interpretations/Limitations** section are included in Appendix A.
  - Where applicable, include a statement regarding the statistical sampling plan used, any inferences that were made, and the confidence level of the inferences.
  - Where applicable, include a statement regarding the limitations when non-statistical sampling was used. Any results shall be limited to the sample(s) that was examined.
  - If relevant isomeric forms of a compound are not differentiated, this will be clearly stated.

- If examinations were limited based on the nature of the evidence (e.g., packaging, quantity, volume, degradation), this will be clearly stated.
  - Define any terminology relevant to the interpretation of a result (e.g., the phrase “consistent with”).
    - i. The terminology "consistent with" does not imply an identification of a specific chemical or product. A substance is termed "consistent with" when the analytical data does not support an identification of a specific chemical or product, but does provide reliable information to include the substance within a class of materials. The phrase "consistent with" is also used when an appropriate reference standard could not be obtained.
- d. The **Remarks** section will include the requirements set forth in the *FBI Laboratory Operations Manual*. The below list contains additional guidance for the **Remarks** section for General Chemistry reports. Examples of appropriate wording for the **Remarks** section are included in Appendix A.
- May include any pertinent chemical or product information.
  - May include relevant controlled substance information (e.g., scheduling, brand names).
  - May include commentary on potential for future examinations if additional items and/or intelligence is gathered.

## 6 Calculations

Not applicable.

## 7 Measurement Uncertainty

Not applicable.

## 8 Limitations

Every scenario cannot be anticipated. This standard operating procedure only serves as a general guideline.



## 9 Safety

Not applicable.

## 10 References

*FBI Approved Standards for Scientific Testimony and Report Language for the General Chemistry Discipline* (GenChem ASSTR)

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

Rev. #	Issue Date	History
1	04/01/11	Revised wording in section 7d.
2	12/02/19	Removed 'subunit' throughout. Changed section 1 to 'Scope' and included applicable personnel. Added reference to 'GenChem ASSTR' and 'GenChem ULTR' in sections 1 and 10. Changed 'Limitations/Interpretations' to 'Interpretations/Limitations' throughout for consistency. Moved 'sampling/inferences' from 'Results of Examinations' to 'Interpretations/Limitations' section (also moved in Appendix). Moved 'product/chemical information' from 'Interpretations/Limitations' section to 'Remarks' (also moved in Appendix). Made edits throughout section 5 for clarity. Added "consistent with" definition to section 5c. Minor edits to section 8 for clarity. Appendices merged into one appendix and renamed as 'A'. Edited previous Appendix content throughout for clarity and consistency, and added additional examples.

**Approval**

Redacted - Signatures on File

Chemistry Unit Chief:

Date: 11/29/2019

General Chemistry  
Technical Leader:

Date: 11/29/2019















## Synthetic Cannabinoid Analysis

### 1 Introduction

Synthetic cannabinoids are manmade compounds that bind to cannabinoid receptors. These compounds began appearing widely in the United States in 2010 in the form of spiked herbal mixtures marketed as incense under a wide variety of product names (e.g., Spice and K2). When the herbal incense is smoked the effects mimic those of marijuana. Redacted

### 2 Scope

This procedure allows for the qualitative identification of the following controlled synthetic cannabinoids in plant material, unknown solids (non-plant material), and drug paraphernalia:

Redacted

Due to the nature of synthetic cannabinoids, compounds not listed above may become of interest. Additional synthetic cannabinoids may be added to this procedure after appropriate validation has been completed.

### 3 Principle

Samples are extracted or rinsed with methanol (or other appropriate solvent). The methanol extract/rinse is analyzed by a combination of the following techniques: direct analysis in real time/time-of-flight mass spectrometry (DART/TOF-MS), gas chromatography/mass spectrometry (GC/MS), and liquid chromatography/mass spectrometry (LC/MS) including MS/MS.

### 4 Specimens

This procedure uses approximately 100 milligrams (mg) of plant material, approximately 1-10 mg of solid (non-plant material), or a methanol rinse of drug paraphernalia.

## 5 Equipment/Materials/Reagents

- a. Volumetric glassware
- b. Adjustable pipetters with disposable tips
- c. Analytical balance
- d. Vortex mixer
- e. Centrifuge
- f. Autosampler vials with screw caps, test tubes, and other common disposable glassware
- g. 0.2 um syringe filters and syringes (or equivalent)
- h. Time-of-Flight Mass Spectrometer with Direct Analysis in Real Time ionization source (DART/TOF-MS) (or equivalent)
- i. Gas Chromatograph/Mass Spectrometer (GC/MS) instrument equipped with electron impact (EI) ionization with a 30 meter HP-5MS [(5%-Phenyl)-methylpolysiloxane] column (or equivalent)
- j. Liquid Chromatograph/Mass Spectrometer (LC/MS) equipped with electrospray ionization (ESI) (capable of high resolution MS and data dependent MS/MS) (or equivalent)
- k. Waters Xterra C18 MS LC column: 100 x 3.0 mm, 3.5 um particle size (or equivalent)
- l. Acetonitrile (HPLC Grade, or equivalent)
- m. Water (HPLC Grade, or equivalent)
- n. Formic Acid (Reagent Grade, or equivalent)
- o. 200 Proof Ethanol (HPLC Grade, or equivalent)
- p. Methanol (HPLC Grade, or equivalent)
- q. LC Mobile Phase A (0.1% Formic Acid in Acetonitrile):  
Add 0.5 mL formic acid to 500 mL acetonitrile. Store in glass at room temperature. Stable

for 2 months.

- r. LC Mobile Phase B (0.1% Formic acid in Water):  
Add 0.5 mL formic acid to 500 mL water. Store in glass at room temperature. Stable for 2 months.

## 6 Standards and Controls

Redacted

Redacted

- i. Synthetic Cannabinoid LC/MS Testmix (1 ug/mL):  
One mL of the Synthetic Cannabinoid HPLC Mixture is diluted to 100 mL with methanol in a volumetric flask. Store in glass <0°C. Stable for at least one year.
- j. Negative Control:  
A solvent (typically methanol) blank from the same source used to prepare the questioned sample(s) serves as the Negative Control for this procedure.
- k. Positive Control (DART/TOF-MS):  
A 100 ppm solution of the synthetic cannabinoid(s) in methanol (or other appropriate solvent) serves as the Positive Control for DART/TOF-MS analysis.
- l. Positive Control (GC/MS):  
A 100 ppm solution of the synthetic cannabinoid(s) in methanol (or other appropriate solvent) serves as the Positive Control for GC/MS analysis.
- m. Positive Control (LC/MS) (1 ug/mL):  
A 1 ppm solution of the synthetic cannabinoid(s) in methanol (or other appropriate solvent) serves as the Positive Control for LC/MS analysis.

## 7 Calibration

Not applicable.

## 8 Sampling

Sampling is performed according to the *Sampling Plan for Controlled Substances, Tablets, Capsules, and Pharmaceuticals*- General Chemistry Subunit SOP Manual.

## 9 Procedure

- a. Accurately record the weight for each specimen.
- b. Perform a visual and microscopic examination of each specimen, where applicable. For a

vegetative specimen, particularly one from within a smoking device, the potential exists for marijuana to be mixed with the specimen. If vegetative material consistent with marijuana is observed, use the procedure entitled *Marijuana Identification- General Chemistry Subunit SOP Manual* in conjunction with this procedure.

- c. For a vegetative specimen, weigh out approximately 100 mg by combining multiple samples taken from different areas of the specimen in order to obtain a representative sample. Transfer the sample to a test tube. If there is insufficient sample, make note of how much sample was weighed out during this step and concentrate the sample per step i. below.
- d. For an unknown solid material, weigh out an appropriate amount (1-10 mg) of the solid into a test tube keeping in mind that the solid may consist of relatively pure synthetic cannabinoid(s).
- e. Add 1 mL methanol (or other appropriate solvent) to the test tube and vortex well. By way of an example, 100 mg of a specimen that consisted of a 1% synthetic cannabinoid concentration by weight would result in a methanolic solution of approximately 1 mg/mL at this point in the procedure.
- f. For a drug paraphernalia specimen, rinse the specimen with an appropriate volume (typically 1 mL) of methanol (or other appropriate solvent) and place the methanol rinse in a test tube. Concentrate the rinse per step i. below.
- g. Centrifuge or filter the methanol extract/rinse.
- h. Transfer the supernatant or filtrate to a new test tube.
- i. If necessary, the methanol extract may be concentrated at this point by evaporating to ~100 uL or to dryness under N<sub>2</sub> gas flow at a temperature of 60 °C. An extract that was taken to dryness will be reconstituted with 100 uL methanol.
- j. Analyze each extract by DART/TOF-MS in the positive ionization mode using the instrumental parameters that follow.
- k. Based on the DART/TOF-MS results and the Limit(s) of Detection (LOD(s)) for the GC/MS analysis of the particular synthetic cannabinoid(s) of interest (see Section 14 b.), prepare the appropriate dilution(s) of the extract in methanol.
- l. Analyze each extract by GC/MS using the instrumental parameters that follow.

- m. If necessary, the extract may be analyzed by LC/MS and/or LC/MS/MS using the instrumental parameters that follow. The extract should be further diluted to achieve a synthetic cannabinoid concentration of approximately 1-5 ug/mL. Prior to analyzing the sample extract, analyze the appropriate Positive Control(s) or the Synthetic Cannabinoid LC/MS Testmix (see Section 6 h.-i.) to ensure that the instrument is operating properly.

Note: The instrumental methods may be performed in a different order, if desired.

## 10 Instrumental Conditions

### 10.1 DART/TOF-MS Parameters

The following DART/TOF-MS parameters may vary slightly among the different DART/TOF-MS instruments. The analysis of appropriate Positive Control(s) will be conducted and verified to provide the correct accurate mass data to ensure that the DART/TOF-MS parameters are suitably set.

#### 10.1.1 DART Ionization Source Parameters

Anode Polarity:	Positive (+)
Needle Voltage:	3999 V
Electrode #1 Voltage:	75 V
Electrode #2 Voltage:	150 V
Gas Control:	~ 1.5 LPM
Temperature Control:	set 410°C (actual ~ 400°C)

### 10.1.2 TOF-MS Parameters

Tune File:	DART + (DART positive)
Needle Voltage:	0 V
Ring Lens Voltage:	5 V
Orifice 1 Voltage:	30 V
Orifice 2 Voltage:	5 V
Orifice 1 Temp:	80 °C
Peaks Voltage:	800 V
Bias Voltage:	25 V
Pusher Bias Voltage:	-0.65 V
Focus Voltage:	-124 V
Focus Lens Voltage:	10.0 V
Quadrupole Lens Voltage:	20.0 V
Right/Left Voltage:	-0.6 V
Top/Bottom Voltage:	-3.0 V
Reflectron Voltage:	950 V
Mass Range:	80-800 <i>m/z</i>

## 10.2 GC/MS Parameters

### 10.2.1 Gas Chromatograph Parameters

Oven		Column		Inlet and Carrier	
Initial Temp.	60 °C	Type	HP-5MS	Inlet Temp.	280 °C
Initial Time	2.00 min.	Length	30.0 m	Injection Volume	1 uL
Ramp Rate	35 °C/min.	Internal Dia.	0.25 mm	Injection Mode	Split
Final Temp.	290 °C	Film Thick.	0.25 um	Split Ratio	30:1
Final Time	25 min.			Split Flow	~ 35 mL/min.
Run Time	33.57 min.			Carrier Gas	He (ultrapure)

### 10.2.2 Mass Spectrometer Parameters – GC/MS

Ionization Mode	EI (+)	Source Temp.	230 °C
Acquisition Mode	Scan	Transfer Line Temp.	280 °C
Scan range	43-560 <i>m/z</i>	Quadrupole Temp.	150 °C
EM Offset	106	Solvent Delay	3.0 min.

### 10.3 LC/MS(/MS) Parameters

#### 10.3.1 Liquid Chromatograph Parameters

Mobile Phase Compositions	Flow Parameters			Column Parameters			
A: 0.1% Formic Acid in acetonitrile	Flow Rate: 0.25 mL/min			Type	Waters Xterra C18 MS		
	Time (min)	%A	%B	Dimensions	10 cm x 3.0 mm		
	initial	30	70				
	3	30	70	Particle Size	3.5 um		
B: 0.1% Formic Acid in water	15	90	10				
	30	90	10			Temperature	30 °C
	31	30	70				
	36	30	70				
Total Run Time: 37 min							

#### 10.3.2 Mass Spectrometer Parameters – LC/MS High Resolution (Resolution = 30,000)

Ionization Mode	Electrospray (+)
Scan Mode	FTMS res=30000
Scan Range	250 650 $m/z$
All source parameters are set through the instrument tune file. Ensure the proper tune file, “spice” is loaded.	

#### 10.3.3 Mass Spectrometer Parameters – LC/MS/MS

Scan Event #1		Scan Event #2	
Ionization Mode	Electrospray (+)	Ionization Mode	Electrospray (+)
Scan Mode	FTMS res=7500	Scan Mode	FTMS res=7500; MS/MS DDS
Scan Range	250 650 $m/z$	Precursor	Most intense of $m/z$ 319.26, 328.17, 333.28, 342.19, 385.19, 387.29
All source parameters are set through the instrument tune file. Ensure the proper tune file, “spice” is loaded.		Isolation Width	2.0
		Collision Energy	35%
		Activation Q	0.250
		Activation Time	30.0
		Scan Range	software control



## **11 Decision Criteria**

### **11.1 DART/TOF-MS**

The M+1 ion of the questioned sample should be within 0.005 amu of the expected M+1 (see Table 1) and/or within 0.005 amu of a contemporaneously analyzed Positive Control.

### **11.2 GC/MS**

#### **11.2.1 Chromatography**

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. Additionally, the following two criteria should be met.

##### **11.2.1.1 Retention Time**

The retention time of the peak should be within  $\pm 5\%$  of the retention time (relative or absolute, as appropriate) obtained from injection of a contemporaneously analyzed Positive Control.

##### **11.2.1.2 Signal-to-Noise**

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio should exceed 3. Further, the baseline signal for the peak of interest should be at least 10-fold greater than that for any observed peak at a similar retention time in a Negative Control or solvent blank injected just prior to the sample.

#### **11.2.2 Mass Spectrometry**

The mass spectrum of the analyte of interest should compare favorably with that of a contemporaneously analyzed Positive Control within a reasonable degree of scientific certainty. The ratios of the fragmented ions (see Table 1) should be compared following the *Guidelines for Comparison of Mass Spectra* - Toxicology Subunit SOP Manual.

### **11.3 LC/MS(/MS)**

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. In general, compound identification should be based on a comparison of the chromatography and mass spectrometry for the analyte peak of interest with data from a contemporaneously analyzed Positive Control.

### 11.3.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. Additionally, the following two criteria should be met.

#### 11.3.1.1 Retention Time

The retention time of the peak should be within  $\pm 5\%$  of the retention time (relative or absolute, as appropriate) obtained from injection of a contemporaneously analyzed Positive Control.

#### 11.3.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio should exceed 3. Further, the baseline signal for the peak of interest should be at least 10 fold greater than that for any observed peak at similar retention time in a Negative Control or solvent blank injected just prior to the sample.

### 11.3.2 Mass Spectrometry

The mass spectrum of the analyte of interest should match that of a contemporaneously analyzed Positive Control within a reasonable degree of scientific certainty. When performing LC/MS (high resolution) the M+1 of the questioned sample should be within 0.005 amu of the expected M+1 (see Table 1) and/or within 0.005 amu of a contemporaneously analyzed Positive Control. When performing LC/MS/MS (data dependant scanning) analyses, the ratios of the product ion(s) (see Table 1) should be compared following the *Guidelines for Comparison of Mass Spectra* - Toxicology Subunit SOP Manual.

Table 1: Synthetic Cannabinoid Exact Mass, EI Fragments, and MS/MS Fragments

Redacted

## 12 Calculations

Not applicable.

## 13 Uncertainty of Measurement

Not applicable.

## 14 Limitations

Redacted

### 14.1.2 Interferences

This procedure is unable to differentiate the enantiomeric pair HU-210/HU-211.

## 15 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

## 16 References

*Marijuana Identification* (GenChem 8); FBI Laboratory Chemistry Unit – General Chemistry Subunit SOP Manual.

*Sampling Plan for Controlled Substances, Tablets, Capsules, and Pharmaceuticals* (GenChem 21); FBI Laboratory Chemistry Unit - General Chemistry Subunit SOP Manual.

*Guidelines for Comparison of Mass Spectra* (Tox 104); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

*FBI Laboratory Safety Manual.*

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Uchiyama, N.; Kikura-Hanajiri, R; Ogata, J. and Goda, Y. Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. *Forensic Science International*, 198: 31-38 (2010). (Tox Database 2032)

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Nekhoroshev, S. V.; Nekhoroshev, V. P.; Remizova, M. N.; and Nekhorosheva, A. V. Determination of the Chemical Composition of *Spice* Aromatic Smoking Blends by Chromatography-Mass Spectrometry. *Journal of Analytical Chemistry*, 66: 1196-1200 (2011).

Kneisel, S.; Westphal, F.; Moosmann, B.; et al. Cannabimimetics II: Mass spectra and ATR-IR spectra of new compounds between the end of 2010 and late 2011. *TIAFT Bulletin*, 41(3): 29-39 (2011).

Rev. #	Issue Date	History
0	02/16/12	New document.

**Approval**

Redacted - Signatures on File

## **Analysis of Phosphodiesterase Type 5 (PDE-5) Inhibitor Drugs**

### **1 Introduction**

The class of drugs known as synthetic phosphodiesterase type 5 (PDE-5) inhibitors is prescribed as a treatment for erectile dysfunction (ED) in males. Sildenafil citrate (Viagra<sup>®</sup>, Pfizer), tadalafil (Cialis<sup>®</sup>, Eli Lilly), and vardenafil hydrochloride (Levitra<sup>®</sup>, Bayer) are PDE-5 inhibitor drugs that are marketed and approved for ED treatment in the United States.

The popularity of these drugs has resulted in a black market trade, where diverted and counterfeit PDE-5 inhibitor drugs are sold. Additionally, some herbal dietary supplements marketed as being effective for the treatment of ED have been shown to contain synthetic PDE-5 inhibitors. These spiked herbal dietary supplements may cause serious medical effects if consumed by someone that should not be prescribed PDE-5 inhibitors.

This procedure may be used to analyze samples for the presence of the PDE-5 inhibitor drugs listed in Appendix A. The combination of tablet logo searching, Fourier-transform infrared (FTIR) spectroscopy, direct analysis in real time/time-of-flight mass spectrometry (DART/TOF-MS), and liquid chromatography/electrospray ionization mass spectrometry (LC/MS(ESI)) may be used to determine the presence or absence of some PDE-5 inhibitor drugs within tablets or other matrices.

### **2 Scope**

This procedure allows for the identification of sildenafil, tadalafil, vardenafil, udenafil, avanafil, and hydroxyhomo sildenafil within tablets/capsules. Analysis for the presence of other PDE-5 inhibitor drugs will require validation before being analyzed by this procedure. Additionally, analysis of non-tablet matrices (e.g., liquids) will require further matrix validation studies before being analyzed by this procedure.

### **3 Principle**

After visual inspection and logo searching (if applicable), samples are homogenized and analyzed by FTIR spectroscopy and/or DART/TOF-MS. Samples are then diluted to an appropriate concentration before confirmation by LC/MS(ESI) analysis.

## 4 Specimens

This procedure is used to analyze samples suspected of containing PDE-5 inhibitor drugs.

## 5 Equipment/Materials/Reagents

- a. Liquid Chromatograph/Mass Spectrometer (ion trap) operating in the electrospray ionization mode (LC/MS(ESI)) equipped with a C18 column (150 mm x 2.1 mm x 5 um particle column) (or equivalent)
- b. Fourier-Transform Infrared (FTIR) Spectrophotometer with Attenuated Total Reflectance (ATR) attachment (or equivalent)
- c. Time-of-Flight Mass Spectrometer with Direct Analysis in Real Time ionization source (DART/TOF-MS) (or equivalent)
- d. Analytical balance
- e. Routine laboratory supplies, including disposable glass pipets, spatulas, autosampler vials, test tube racks, graduated cylinders, syringes, syringe filters, etc.
- f. Acetonitrile (Optima grade or equivalent)
- g. Water (Optima grade or equivalent)
- h. Formic Acid (Reagent grade or equivalent)
- j. Mobile Phase A consisting of 0.1 % Formic Acid (aqueous). Add 250 uL Formic Acid to an appropriate, volumetric glass container. Bring to 250 mL mark with deionized H<sub>2</sub>O and mix well.
- k. Mobile Phase B consisting of 100 % acetonitrile (CH<sub>3</sub>CN).
- l. Sildenafil citrate (Pfizer, reagent grade or equivalent)
- m. Tadalafil (Eli Lilly, reagent grade or equivalent)
- n. Vardenafil hydrochloride (Bayer, reagent grade or equivalent)
- o. Udenafil (Dong-A Pharmaceutical, reagent grade or equivalent)



- p. Avanafil (Toronto Research Chemicals, reagent grade or equivalent)
- q. Hydroxyhomo sildenafil (Toronto Research Chemicals, reagent grade or equivalent)

## 6 Standards and Controls

- a. FTIR:  
The PDE-5 inhibitor drug in question will be analyzed as a Positive Control on the FTIR.
- b. DART/TOF-MS:  
The PDE-5 inhibitor drug in question with a concentration of at least 100 ug/mL (in an appropriate solvent, CH<sub>3</sub>CN for example) will be analyzed as a Positive Control on the DART/TOF-MS.
- c. LC/MS(ESI):  
A solution of the PDE-5 inhibitor drug in question with a concentration of 1-10 ug/mL (in an appropriate solvent, CH<sub>3</sub>CN for example) will be analyzed as a Positive Control on the LC/MS(ESI).
- d. Negative Control:  
In most instances, a solvent blank from the same source used to prepare the questioned sample(s) will serve as an appropriate Negative Control. For non-tablet matrices, a Negative Control should be prepared in a matrix that mimics the questioned sample(s). It is left to the discretion of the examiner as to what constitutes an adequate Negative Control, keeping in mind good laboratory practices and quality assurance guidelines.

## 7 Calibration

Not applicable.

## 8 Sampling

Sampling is performed according to the *Sampling Plan for Controlled Substances, Tablets, Capsules, and Pharmaceuticals*- General Chemistry Subunit SOP Manual.

## 9 Procedure

- a. Visually examine each specimen and where applicable compare any logos/imprints to the information in Table 2 (Section 11.3) or another valid reference source.
- b. Record the weight of each specimen using an appropriate analytical balance.
- c. Select samples for further analysis by utilizing the *Sampling Plan for Controlled Substances, Tablets, Capsules, and Pharmaceuticals* (FBI Laboratory Chemistry Unit – General Chemistry Subunit SOP Manual).
- d. Homogenize each tablet sample with a mortar and pestle.
- e. Analyze each sample directly by FTIR (transmission or ATR). Spectral subtraction may be necessary to observe infrared absorptions from the PDE-5 inhibitor drugs. In some preparations (e.g., 5 mg Cialis<sup>®</sup> tablets), the PDE-5 inhibitor drug may not be concentrated enough to observe the infrared absorptions even with spectral subtraction of the excipient(s).
- f. If the PDE-5 inhibitor drug was not detected by FTIR, analyze the sample(s) by DART/TOF-MS in the positive ionization mode. Samples may be analyzed neat or in solution (concentration  $\geq 100$  ug/mL in an appropriate solvent, CH<sub>3</sub>CN for example).
- g. Prepare each sample in CH<sub>3</sub>CN (or in a 25% Mobile Phase B:75% Mobile Phase A solution) to achieve a PDE-5 inhibitor drug concentration of approximately 1-10 ug/mL. Filter each solution with a 0.2 um syringe filter (or equivalent).
- h. Analyze the filtered CH<sub>3</sub>CN solutions by LC/MS(ESI) in the positive ionization mode using the full scan detection mode at a minimum. Additional MS/MS scan events may be added to the full scan event as deemed appropriate.

## 10 Instrumental Conditions

The following instrumental conditions may be used for this analysis:

### 10.1 FTIR Spectrophotometer

Benchtop: Wave numbers: 4000-400 cm<sup>-1</sup>  
Scans: 32

ATR attachment: Wave numbers: 4000-650  $\text{cm}^{-1}$  (or 4000-400  $\text{cm}^{-1}$ )  
 Scans: 32

## 10.2 DART/TOF-MS

The following DART/TOF-MS parameters may vary slightly among the different DART/TOF-MS instruments. The analysis of appropriate Positive Control(s) will be conducted and verified to provide the correct accurate mass data to ensure that the DART/TOF-MS parameters are suitably set.

DART Ionization Source Parameters:

Anode Polarity:	Positive (+)
Needle Voltage:	3999 V
Electrode #1 Voltage:	75 V
Electrode #2 Voltage:	150 V
Gas Control:	~ 1.5 LPM
Temperature Control:	set 410°C (actual ~ 400°C)

TOF-MS Parameters:

Tune File:	DART + (DART positive)
Needle Voltage:	0 V
Ring Lens Voltage:	5 V
Orifice 1 Voltage:	30 V
Orifice 2 Voltage:	5 V
Orifice 1 Temp.:	80°C
Peaks Voltage:	800 V
Bias Voltage:	25 V
Pusher Bias Voltage:	-0.65 V
Focus Voltage:	-124 V
Focus Lens Voltage:	10.0 V
Quadrupole Lens Voltage:	20.0 V
Right/Left Voltage:	-0.6 V
Top/Bottom Voltage:	-3.0 V
Reflectron Voltage:	950 V
Mass Range:	80-800 $m/z$

### 10.3 LC/MS(ESI) Parameters

Mobile Phase Compositions	Flow Parameters			Column Parameters	
A: 0.1% Formic Acid (aq.)	Total Flow: 0.30 mL/min			Type	C18
	Time (min)	%A	%B	Dimensions	15 cm x 2.1 mm
	0.1	75	25		
	5	75	25		
B: 100% CH <sub>3</sub> CN	15	10	90	Particle Size	5 µm
	20	10	90	Temperature	30 °C
	21	75	25		
	27	75	25		
	Total Run Time: 27 min				

Ionization Mode	Electrospray (+)	All source parameters are set through the instrument tune file. Ensure the proper tune file, "sildenafil_475" is loaded.
Scan Modes	Full scan and optional 1-6 scan event MS/MS	
Scan Range	Full Scan: 130.0-550.0 <i>m/z</i> MS/MS: 130.0-550.0 <i>m/z</i> , 35.0% CID CE, Q-0.250, Time-30.0, IsoW- 2.0 Precursor 1- 475.00 [Sildenafil + H] Precursor 2- 390.00 [Tadalafil + H] Precursor 3- 489.00 [Vardenafil + H] Precursor 4- 516.50 [Udenafil + H] Precursor 5- 484.00 [Avanafil + H] Precursor 6- 504.50 [Hydroxyhomo sildenafil + H]	

## 11 Decision Criteria

The following criteria are used as guides in determining the acceptability of the data produced in this assay. In general, a comparison of the response for the analyte peak of interest with data from a contemporaneously analyzed reference standard should meet the following criteria.

### 11.1 Chromatography

- a. Peaks should show good chromatographic characteristics, with reasonable peak shape, width, and resolution.

- b. The retention time of the peak should be within  $\pm 2\%$  of that for a contemporaneously analyzed Positive Control.
- c. The baseline signal-to-peak noise ratio for an analyte should be greater than three (3).
- d. The signal intensity for an analyte peak should be at least ten-times greater than the intensity of any carryover peaks which may have been present in analyses prior to the sample (e.g., Blanks or Negative Controls).

## 11.2 Mass Spectrometry

The mass spectrum of the analyte of interest should compare favorably with that of a contemporaneously analyzed Positive Control within a reasonable degree of scientific certainty. See the Chemistry Unit's *Guidelines for Comparison of Mass Spectra* standard operating procedure (Tox 104-1) for further guidance. Table 1 contains the  $[M+H]^+$  ions that should be observed by DART/TOF-MS and LC/MS(ESI) for the listed PDE-5 inhibitor drugs, as well as some of the observed MS/MS product ions (precursor ion underlined, where applicable).

Table 1: PDE-5 Inhibitor Drugs, Observed Ions

Analyte	DART/TOF-MS, $[M+H]^+$ ( $m/z$ )	LC/MS(ESI) Full Scan, $[M+H]^+$ ( $m/z$ )	LC/MS(ESI), MS/MS ( $m/z$ )
Sildenafil	475.213	475	<u>475</u> , 377, 311, 283
Tadalafil	390.145	390	302, 268, 262
Vardenafil	489.228	489	<u>489</u> , 377, 299, 151
Udenafil	517.260	517	<u>517</u> , 474, 418, 325
Avanafil	484.186 (CI-35) 486.184 (CI-37)	484 (CI-35) 486 (CI-37)	375, 349, 221
Hydroxyhomo sildenafil	505.223	505	487, 461, 377

## 11.3 All Other Tests

All comparison tests (e.g., visual inspections, FTIR, etc.) should compare favorably to the corresponding Positive Control. See Table 2 for Viagra<sup>®</sup>, Cialis<sup>®</sup>, and Levitra<sup>®</sup> tablet information.

Table 2: Prescription Tablet Information

Tablet	Color	Shape	Side 1	Side 2
Viagra <sup>®</sup> , 100 mg	Blue	Diamond	<i>Pfizer</i>	VGR 100
Viagra <sup>®</sup> , 50 mg				VGR 50
Viagra <sup>®</sup> , 25 mg				VGR 25
Cialis <sup>®</sup> , 20 mg	Yellow	Ovoid	C 20	none
Cialis <sup>®</sup> , 10 mg			C 10	
Cialis <sup>®</sup> , 5 mg			C 5	
Cialis <sup>®</sup> , 2.5 mg			C 2 ½	
Levitra <sup>®</sup> , 20 mg	Orange	Round	B A Y E R	20
Levitra <sup>®</sup> , 10 mg				10
Levitra <sup>®</sup> , 5 mg				5
Levitra <sup>®</sup> , 2.5 mg				2.5

## 12 Calculations

Not applicable.

## 13 Uncertainty of Measurement

Not applicable.

## 14 Limitations

- a. Limit of Detection: DART/TOF-MS  $\leq 100$  ug/mL, LC/MS(ESI)  $\leq 1$  ug/mL
- b. Interferences: Sildenafil, Avanafil, and Hydroxyhomo sildenafil have similar LC/MS(ESI) retention times and share some of the same full scan ions (e.g., 312 and 284  $m/z$ ). These ion interferences can be resolved through the use of MS/MS.

## 15 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.

## 16 References

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Gratz, S.R., Flurer, C.L., and Wolnik, K.A., "Analysis of undeclared synthetic phosphodiesterase-5 inhibitors in dietary supplements and herbal matrices by LC-ESI-MS and LC-UV", *Journal of Pharmaceutical and Biomedical Analysis* 36, (2004), pp 525-533.

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Singh, S., Prasad, B., Savaliya, A.A., et. al., "Strategies for characterizing sildenafil, vardenafil, tadalafil and their analogues in herbal dietary supplements, and detecting counterfeit products containing these drugs", *Trends in Analytical Chemistry*, Vol. 28, No. 1, 2009, pp 13-28.

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*Performance Monitoring Protocol (QA/QC) for the Nicolet FTIRs*; FBI Laboratory Chemistry Unit – Instrument Operation and Support Subunit SOP Manual.

*Performance Monitoring Protocol (QA/QC) for the JEOL AccuTOF DART*; FBI Laboratory Chemistry Unit – Instrument Operation and Support Subunit SOP Manual.

*Performance Monitoring Protocol (QA/QC) for the Thermo LTQ LC/MS (ESI)*; FBI Laboratory Chemistry Unit - Instrument Operation and Support Subunit SOP Manual.

*Sampling Plan for Controlled Substances, Tablets, Capsules, and Pharmaceuticals*; FBI Laboratory Chemistry Unit – General Chemistry Subunit SOP Manual.

*Guidelines for Comparison of Mass Spectra*; FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

*FBI Laboratory Safety Manual*.



Rev. #	Issue Date	History
0	12/05/11	Original document.

**Approval**

Redacted - Signatures on File

**Appendix A. PDE-5 Inhibitor Drugs Chemical Information**

Redacted - Form on File

## **Insulin Analysis by LC-ESI-MS**

### **1 Introduction**

Insulin is an endogenous protein hormone. It is secreted by the pancreas to regulate blood glucose levels. Insulin is also available commercially for the treatment of diabetes. Commercial insulin preparations include human, bovine, and porcine forms, as well as various synthetic formulations.

Human insulin (MW 5808), porcine insulin (MW 5778) and bovine insulin (MW 5734) differ by a few amino acids. There are synthetic insulins available that also differ from human insulin by a few amino acids. Examples include Apidra (MW 5823), Lantus (MW 6063), Levemir (MW 5917), and Novolog (MW 5826). The synthetic insulin Humalog is composed of exactly the same amino acids as human insulin, but arranged in a slightly different sequence. Additionally, there are several recombinant insulin preparations, including Humulin and Novolin.

Since proteins can carry multiple charges in solution, multiple charge states of the various types of insulin can be examined using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS).

### **2 Scope**

This procedure allows for the detection of human, bovine, and porcine insulin, as well as several synthetic insulins, in pharmaceutical preparations, syringe residues, solids, and non-biological liquids.

### **3 Principle**

Liquid samples are diluted to an appropriate concentration before analysis. Solids and residues are dissolved in dilute acetic acid before analysis. Separation is performed using a C18 column. Detection is by LC-MS-ESI, at either unit mass resolution on an ion trap instrument or at 30,000 resolution on a Fourier transform instrument.

### **4 Specimens**

This procedure may be used to analyze pharmaceutical preparations, syringe residues, solids, and other non-biological liquids.

## 5 Equipment/Materials/Reagents

- a. Liquid Chromatograph / Mass Spectrometer (linear ion trap and/or Orbitrap) operating in the positive electrospray ionization mode
- b. Vydac 218TP (or equivalent) C-18 liquid chromatography column (2.1x150 mm; 5  $\mu$ m particle size) with a 2.1x7.5 mm guard cartridge.
- c. Balance accurate to at least  $\pm 0.2$  mg
- d. Adjustable pipetters (0.005 mL to 1 mL) with appropriate tips
- e. 1 mL disposable plastic syringes with needles
- f. Volumetric flasks (5, 10, and 25 mL)
- g. Screw-top borosilicate glass test tubes. (Thermo-Fisher Scientific part number 14-959-35AA, or equivalent). See limitations section for use of alternate test tubes.
- h. Screw-top glass autosampler vials (Microliter Analytical Supplies, Inc. part number 09-1220B-101, or equivalent) with low volume inserts removed. See limitations section for use of alternate vials.
- i. Routine laboratory supplies, including disposable glass pipets, spatulas, test tube racks, graduated cylinders, etc.
- j. Acetonitrile (Optima grade or better)
- k. Water (both deionized (DI) and Optima grade)
- l. Dilute Trifluoroacetic Acid (TFA) (0.04% by volume)  
Add 0.4 mL TFA (Puriss grade) to 1 L water (Optima grade or better) and mix well. Store in glass at room temperature. Stable for at least two months.
- m. Dilute acetic acid (50mM)  
To a 100 mL graduated cylinder add 80 mL DI water and 250  $\mu$ L glacial acetic acid (ACS grade or better). Bring to 85 mL with DI water and mix well. Store in glass at room temperature. Stable for at least 3 months.

- n. Dilute hydrochloric acid (0.1M)  
To a 25 mL graduated cylinder add 20 mL DI water followed by 2 mL concentrated hydrochloric acid (ACS grade or better). Mix well and bring to 25 mL with DI water. Store in glass at room temperature. Stable for at least 6 months.
- o. ProMass for Xcalibur (Automated ESI Deconvolution and Web-based Reporting software for Finnigan Xcalibur) (or equivalent)

## 6 Standards and Controls

- a. Human Insulin:  
Purchased from United States Pharmacopeia (USP) or another approved vendor. Stability and storage determined by manufacturer.
- b. Porcine Insulin:  
Purchased from USP or another approved vendor. Stability and storage determined by manufacturer.
- c. Bovine Insulin:  
Purchased from USP or another approved vendor. Stability and storage determined by manufacturer.
- d. Human Insulin Stock Solution (1 mg/mL):  
Weigh 10.0 mg Human Insulin into a 10-mL volumetric flask. Add 0.5 mL dilute hydrochloric acid and mix well to dissolve. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable at least six months.
- e. Human Insulin Working Solution (50 µg/mL):  
Add 0.5 mL Human Insulin Stock Solution to a 10-mL volumetric flask. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable at least six months.
- f. Porcine Insulin Stock Solution (1 mg/mL):  
Weigh 10.0 mg Porcine Insulin into a 10-mL volumetric flask. Add 0.5 mL dilute hydrochloric acid and mix well to dissolve. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable at least six months.
- g. Porcine Insulin Working Solution (50 µg/mL):  
Add 0.5 mL Porcine Insulin Stock Solution to a 10-mL volumetric flask. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable at least six months.

months.

- h. Bovine Insulin Stock Solution (1 mg/mL):  
Weigh 10.0 mg Bovine Insulin into a 10-mL volumetric flask. Add 0.5 mL dilute hydrochloric acid and mix well to dissolve. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable at least six months.
- i. Bovine Insulin Working Solution (50 µg/mL):  
Add 0.5 mL Bovine Insulin Stock Solution to a 10-mL volumetric flask. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable at least six months.
- j. System Suitability Standard Solution (5 µg/mL):  
Add 125 µL each of the Human, Porcine, and Bovine Insulin Stock Solutions to a 25-mL volumetric flask. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable at least six months. A portion of this standard will be analyzed with every insulin assay.
- k. Humalog Insulin Lispro Injection Solution (100 units/mL)  
Obtained from Eli Lilly & Company. Storage and stability determined by manufacturer.
- l. Humalog Insulin working Solution (approximately 52 µg/mL)  
Using a 1 mL disposable plastic syringe, transfer 0.15 mL of the Humalog Insulin Lispro Injection Solution to a 10-mL volumetric flask and bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable at least six months, or until expiration of the original solution, whichever comes first.
- m. Novolog Insulin Aspart Injection Solution (100 units/mL)  
Obtained from Novo-Nordisk. Storage and stability determined by manufacturer.
- n. Novolog Insulin working Solution (approximately 52 µg/mL)  
Using a 1 mL disposable plastic syringe, transfer 0.15 mL of the Novolog Insulin Aspart Injection Solution to a 10-mL volumetric flask and bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable at least six months, or until expiration of the original solution, whichever comes first.
- o. Levemir Insulin Detemir Injection Solution (100 units/mL)  
Obtained from Novo-Nordisk. Storage and stability determined by manufacturer.
- p. Levemir Insulin Working Solution (approximately 142 µg/mL)  
Using a 1 mL disposable plastic syringe, transfer 0.1 mL of the Levemir Insulin Detemir

Injection Solution to a 10-mL volumetric flask and bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable at least six months, or until expiration of the original solution, whichever comes first.

- q. Lantus Insulin Glargine Injection Solution (100 units/mL)  
Obtained from Sanofi-Aventis. Storage and stability determined by manufacturer.
- r. Lantus Insulin Working Solution (approximately 55  $\mu\text{g/mL}$ )  
Using a 1 mL disposable plastic syringe, transfer 0.15 mL of the Lantus Insulin Glargine Injection Solution to a 10-mL volumetric flask and bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable at least six months, or until expiration of the original solution, whichever comes first.
- s. Synthetic Insulins Standard Solution (approximately 5  $\mu\text{g/mL}$  of each component)  
To a 5-mL volumetric flask, add 480  $\mu\text{L}$  each of the Humalog and Novolog Insulin Working Solutions, 175  $\mu\text{L}$  of the Levemir Insulin Working Solution, and 450  $\mu\text{L}$  of the Lantus Insulin Working Solution. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least one month. If needed or desired, this solution may be prepared with only some of the four listed synthetic forms of insulin.
- t. Apidra Insulin Glulisine Injection Solution (100 units/mL)  
Obtained from Sanofi-Aventis. Storage and stability determined by manufacturer.
- u. Apidra Insulin Working Solution (approximately 52  $\mu\text{g/mL}$ )  
Using a 1 mL disposable plastic syringe, transfer 0.15 mL of the Apidra Insulin Glulisine Injection Solution to a 10-mL volumetric flask and bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable at least six months, or until expiration of the original solution, whichever comes first.
- v. Apidra Insulin Standard Solution (approximately 10  $\mu\text{g/mL}$ )  
Add 960  $\mu\text{L}$  of the Apidra Insulin Working Solution to a 5-mL volumetric flask and bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least one month.
- w. Negative Control:  
Dilute acetic acid is used as a negative control for this procedure. A Negative Control will be analyzed with every insulin assay.

## 7 Calibration

Not applicable.

## 8 Sampling

Not applicable.

## 9 Procedure

- a. Dilute liquid samples with an appropriate amount of dilute acetic acid. For suspected pharmaceutical preparations, a 200-fold dilution (50  $\mu$ L of sample to a final volume of 10 mL) is recommended. For fluids recovered from IV drip bags, a 10-fold dilution (200  $\mu$ L of sample plus 1.8 mL of dilute acetic acid) is recommended. Dilution factors for other samples will depend on case history and professional judgment.
- b. Accession a 1 mL portion of the diluted sample and add 20  $\mu$ L of the Bovine Insulin Stock Solution as internal standard. Alternatively, add 20  $\mu$ L of the Porcine Insulin Stock Solution if analysis of bovine insulin is desired. Prepare 1 mL portions of appropriate control solutions as described in section 6 of this procedure and spike each with 20  $\mu$ L of the chosen internal standard solution.
- c. Verify that the LC/MS is in proper working condition by analyzing the System Suitability Standard Solution using the instrumental operating conditions in Section 10 of this procedure. The results can be compared to previous chromatograms of the control solution.
- d. Transfer samples and controls to autosampler vials and analyze 10  $\mu$ L by LC/MS.
- e. Process applicable mass spectra using ProMass for Xcalibur ESI deconvolution software.
  1. Export the desired mass spectrum from the Xcalibur Qual Browser (right click, Export- Clipboard- Exact Mass).
  2. Open ProMass for Xcalibur and click on the "Build Params" icon.
  3. Load the "INSULIN.PARAMS" file.
  4. Click on the clipboard icon, which will run the deconvolution program. A report containing the Base Peak Mass, Intensity, and Spectral Quality will be generated.



## 10 Instrumental Conditions

Following are the instrumental conditions to be used in this procedure.

### 10.1 Liquid Chromatography Parameters

Mobile Phase Compositions	Flow Parameters			Column Parameters	
A: dilute TFA (0.04%)	total flow	0.3 mL/min		type	C 18
	time (min)	%A	%B	length	15 cm
B: acetonitrile	0	75	25	internal diameter	2.1 mm
	2	75	25	particle size	5 µm
	5	71	29	temperature	30°C
	12	70	30	Autosampler	
	16	20	80	temperature	20°C
	18	20	80		
	19	75	25		
	24	75	25		
	total run time		24 min		

### 10.2 Mass Spectrometer Parameters

ionization mode	electrospray (+)	sheath gas flow	45 (arbitrary units)
scan mode	full scan	aux gas flow	20 (arbitrary units)
scan range (LTQ)	950 – 2000 m/z	sweep gas flow	0 (off)
resolving power (Orbitrap)	30,000 (nominal)	capillary temperature	300°C
scan range (OrbiTrap)	1400 – 2060 m/z	capillary voltage	60 V
data collection time	24 min.	tube lens voltage	175 V

## 11 Decision Criteria

The following criteria are used as guides in determining the acceptability of the data produced in this assay. In general, a comparison of the chromatography and mass spectrometry for the analyte peak of interest with data from a contemporaneously analyzed reference standard or extracted positive control should meet the following criteria.

## 11.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

### 11.1.1 Retention Time

The retention time of the peak should be within  $\pm 5\%$  of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard or other appropriate positive control.

### 11.1.2 Signal-to-Noise

To justify the existence of a peak, its signal to noise ratio should exceed 3. For high-resolution mass spectral data, automated signal to noise calculators may not perform properly, and the signal to noise ratio may be evaluated visually. Further, the baseline corrected signal for the peak from the sample of interest should be at least 10 fold greater than that for any observed peak at a similar retention time in a negative control injected prior to that sample.

## 11.2 Mass Spectrometry

The mass spectrum (ESI and Deconvoluted) of the analyte of interest should match that of the appropriate reference standard within a reasonable degree of scientific certainty. See the *Guidelines for Comparison of Mass Spectra* standard operating procedure (Tox 104) for further guidance. Table 1 lists the ions that should be observed for each type of insulin at unit mass resolution. Table 2 shows the ions that should be observed ( $\pm 0.006$  m/z tolerance) for each type of insulin at 30,000 resolution.

Table 1: Insulin +5, +4, and +3 Ions(\*) at Unit Mass Resolution

Type of Insulin	Ions Observed (m/z)
Human Insulin and Humalog (insulin lispro)	1162-1164, 1452-1454, 1936-1938
Porcine Insulin	1156-1158, 1444-1446, 1926-1928
Bovine Insulin	1147-1149, 1433-1435, 1911-1913
Apidra (insulin glulisine)	1165-1167, 1456-1458, 1941-1943
Lantus (insulin glargine)	1011-1013, 1213-1215, 1516-1518(*)
Levemir (insulin detemir)	1183-1185, 1479-1481, 1972-1974
Novolog (insulin aspart)	1165-1167, 1456-1458, 1942-1944

\* +6, +5, and +4 ions for Lantus

Table 2: Insulin +4 and +3 Ions at 30,000 Resolution

Human Insulin and Humalog (insulin lispro)	1452.167	1935.887	48.6
	1452.418	1936.222	82.7
	1452.668	1936.556	100
	1452.919	1936.889	95.5
	1453.169	1937.223	76.3
	1453.420	1937.557	52.7
Porcine Insulin	1444.665	1925.884	48.9
	1444.915	1926.218	83.0
	1445.166	1926.552	100
	1445.416	1926.886	95.2
	1445.667	1927.220	75.8
	1445.917	1927.554	52.2
Bovine Insulin	1433.658	1911.209	49.5
	1433.909	1911.543	83.5
	1434.159	1911.877	100
	1434.410	1912.211	94.6
	1434.660	1912.544	74.9
	1434.910	1912.878	51.4
Apidra (insulin glulisine)	1455.917	1940.887	48.4
	1456.168	1941.221	82.5
	1456.418	1941.555	100
	1456.669	1941.889	95.7
	1456.919	1942.223	76.6
	1457.170	1942.557	53.1
Lantus (insulin glargine)	1515.963	2020.948	45.3
	1516.213	2021.282	80.1
	1516.464	2021.616	100
	1516.714	2021.950	98.4
	1516.965	2022.284	80.9
	1517.215	2022.617	57.4
Levemir (insulin detemir)	1479.455	1972.271	46.1
	1479.706	1972.605	80.7
	1479.956	1972.939	100
	1480.207	1973.273	97.6
	1480.457	1973.607	79.6
	1480.707	1973.941	56.1

Novolog (insulin aspart)	1456.661	1941.879	48.8
	1456.912	1942.213	82.8
	1457.162	1942.547	100
	1457.412	1942.881	95.4
	1457.663	1943.215	76.2
	1457.913	1943.549	52.7

## 12 Calculations

Not applicable.

## 13 Uncertainty of Measurement

Not applicable.

## 14 Limitations

a. Limits of Detection:

Table 3 – Limits of detection in samples diluted for analysis: Matrix limit of detection will depend on the dilution factor actually used.

Type of Insulin	LOD (unit resolution)	LOD (high resolution)
Human Insulin	3 µg/mL	2 µg/mL
Porcine Insulin	3 µg/mL	2 µg/mL
Bovine Insulin	3 µg/mL	2 µg/mL
Humalog (insulin lispro)	3 µg/mL	2 µg/mL
Apidra (insulin glulisine)	10 µg/mL	2 µg/mL
Lantus (insulin glargine)	3 µg/mL	3 µg/mL
Levemir (insulin detemir)	2 µg/mL	2 µg/mL
Novolog (insulin aspart)	3 µg/mL	2 µg/mL

b. Interferences: No interferences were observed for blank 10-fold dilutions of IV sterile saline, IV potassium chloride, IV dextrose, or IV metronidazole. The only observed interferences from the target analytes are for human insulin with Humalog and for Novolog with Apidra. Human insulin and Humalog are mass spectrometrically indistinguishable and exhibit only partial chromatographic resolution via this procedure. Thus the presence of high levels of one of these analytes could mask the presence of low levels of the other and simultaneous

presence of high levels of both analytes might preclude the identification of either. Apidra and Novolog cannot be distinguished by this procedure at unit mass resolution. At high mass resolution, this method is able to partially resolve the mass spectra of the two compounds, but presence of high levels of one analyte might mask presence of low levels of the other, and presence of high levels of both compounds might preclude identification of either.

- c. Carryover: For all of the validated compounds except Levemir (insulin detemir), carryover following injection of a 100 µg/mL standard was less than 0.2%. For Levemir, approximately 1% carryover was observed following injection of a 100 µg/mL standard.
- d. Stability: During method validation, it was observed that certain glass surfaces caused rapid loss or degradation of insulins in solution. Stock solutions stored in the specific test tubes listed in 5(g) were observed to be stable for more than five months. The specific vials listed in 5(h) were used for the validation study and do not produce significant insulin loss over a 72 hour period. Other test tubes or vials may be used if a stability study is performed first.

## 15 Safety

Take standard precautions for the handling of chemicals, potentially infectious materials, and hypodermic needles. See the *FBI Laboratory Safety Manual* for guidance.

## 16 References

Darby, S.M., Miller, M.L., Allen, R.A., and LeBeau, M.A., A Mass Spectrometric Method for Quantitation of Intact Insulin in Blood Samples", *Journal of Analytical Toxicology* 25: 8-14 (2001).

*Guidelines for Comparison of Mass Spectra* (Tox 104); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

FBI Laboratory Chemistry Unit - Instrument Operation and Support Subunit SOP Manual.

*FBI Laboratory Safety Manual*.

Rev. #	Issue Date	History
0	11/08/12	New document derived from previous toxicology SOP titled, <i>Insulin Analysis by LC/MS(ESI)</i> .

**Approval**

Redacted Signatures on File

## **Chemistry Unit (CU)**

# **FBI Approved Standards for Scientific Testimony and Report Language for General Chemistry**

### **1 Purpose**

This document provides examples of the statements approved for reporting examination conclusions and offering expert opinion statements during testimony by General Chemistry examiners. It is noted that these examples are not intended to be all inclusive and may be dependent upon the precedent set by the judge or locality in which a testimony is provided. Further, these examples are not intended to serve as precedent for other forensic laboratories and do not imply that statements by other forensic laboratories are incorrect, indefensible, or erroneous.

### **2 Scope**

This document applies to Chemistry Unit (CU) employees who prepare *Laboratory Reports* and/or provide expert testimony in the General Chemistry group. This document does not apply to CU employees who provide fact witness testimony.

### **3 Responsibilities**

**3.1** The examiner will ensure that a *Laboratory Report* complies with the approved statements contained within this document and the *Department of Justice Uniform Language for Testimony and Reports for General Forensic Chemistry and Seized Drug Examinations* document (GenChem ULTR).

**3.2** The examiner will ensure that his or her testimony is consistent with the standards contained within this document and the GenChem ULTR.

**3.3** The Technical and Administrative Reviewers will ensure compliance of General Chemistry *Laboratory Reports* with the statements contained within this document and the GenChem ULTR.

**3.4** An authorized testimony evaluator will assess if General Chemistry testimony complies with the statements contained within this document and the GenChem ULTR.



#### **4 Statements Approved for FBI General Chemistry Testimony and/or Laboratory Reports**

For more detailed guidance on report writing in the General Chemistry group, see the *General Approach to Report Writing* standard operating procedure.

- Identification (i.e., identified)- ‘Identification’ is an examiner’s conclusion that the scientific data supports the presence of an analyte or class of analytes in a questioned sample.
- Consistent with- ‘Consistent with’ is an examiner’s conclusion that the scientific data supports the presence of a questioned sample within a class of materials. The limitation(s) that prevented the assertion of an ‘identification’ conclusion will be communicated.
- Not identified- ‘Not identified’ is an examiner’s conclusion that the scientific data supports the determination that an analyte or class of analytes is not present in a questioned sample or at a detectable level.
- Cannot be differentiated- ‘Cannot be differentiated’ is an examiner’s conclusion that the scientific data does not demonstrate any significant differences between two or more questioned samples that are compared.
- Excluded- ‘Excluded’ is an examiner’s conclusion that the scientific data supports the elimination of a questioned sample as a source of another questioned sample, or that two or more questioned samples do not share a common source.
- Inconclusive- ‘Inconclusive’ is an examiner’s conclusion that the scientific data supports the decision that no determination can be made regarding the questioned sample or the comparisons.
- An examiner may also report and/or state the following supplemental information:
  - a. The weight or volume of a substance which was examined. The weight or volume reported will include an associated estimated measurement uncertainty.
  - b. The potential uses of a substance or class of substances.
  - c. The limitations of his or her examinations and/or opinions.
  - d. The general effects and/or properties of a drug or chemical.

#### **5 Statements Not Approved For FBI General Chemistry Testimony and/or Laboratory Reports**

- If an analyte or class of analytes is identified in a questioned sample, an examiner will not assert how that analyte or class of analytes was transferred to the questioned sample or how long that analyte or class of analytes has been present in the questioned sample.
- When analyzing a portion of a population, an examiner will not assert that his or her conclusion applies to the entirety of the population (or a percentage of the population),

unless a statistically based sampling plan is used. When such a conclusion is offered, the examiner will clearly explain the assertion being made, the results of the sampling unit(s) tested, and the confidence level.

- Except in justified circumstances (e.g., chemical ‘tags’ were incorporated in the questioned sample(s), or the entire population of potential sources was tested) an examiner will not assert the exact source of an analyte or class of analytes. When such an assertion is made, an examiner will explain the circumstances that permit it.
- An examiner will not assert that General Chemistry examinations are infallible or have a zero error rate.
- An examiner will not provide a conclusion that includes a statistic or numerical degree of probability except when based on relevant and appropriate data.
- An examiner will not cite the number of General Chemistry examinations performed in his or her career as a direct measure for the accuracy of a proffered conclusion. (An examiner may cite the number of General Chemistry examinations performed in his or her career for the purpose of establishing, defending, or describing his or her qualifications or experience.)
- An examiner will not use the expressions ‘reasonable degree of scientific certainty,’ ‘reasonable scientific certainty,’ or similar assertions of reasonable certainty in either *Laboratory Reports* or testimony unless required to do so by a judge or applicable law.
- An examiner may not report the purity of a chemical, but can state his or her opinion about an estimated concentration of a chemical as long as it is clearly stated that the estimate is not the result of a validated quantitative measurement.

## 6 Laboratory Report Reviews

The content of a General Chemistry *Laboratory Report* will be reviewed per the *FBI Laboratory Quality Assurance Manual*, *FBI Laboratory Operations Manual*, and *Chemistry Unit Case Record and Review Procedures* ensuring compliance with the approved statements in this document and the GenChem ULTR.

## 7 Testimony Reviews

General Chemistry testimonies will be reviewed following the *FBI Laboratory Practices for Testimony Related Activities*. The review will ensure compliance with the statements in this document and the GenChem ULTR.

## 8 References

*General Approach to Report Writing in the General Chemistry Subunit*

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

*Chemistry Unit Case Record and Review Procedures*

*FBI Laboratory Quality Assurance Manual*

*FBI Laboratory Operations Manual*

Rev. #	Issue Date:	History:
0	05/23/2014	New document.
1	09/12/2019	Changed title to remove “discipline”. Removed “subunit” throughout (except section 8). “ <i>Report of Examination</i> ” changed to “ <i>Laboratory Report</i> ” throughout. Changed “his/her” to “his or her” throughout (consistency with ULTR). Minor formatting edits made in section 2. Integrated GenChem ULTR into section 4 and 5. Updated LOM reference in section 7. Changed format of references in section 8 and removed ASCLD/LAB Supplemental reference.

**Approval**

Redacted - Signatures on File

General Chemistry  
Technical Leader:

Date: 09/11/2019

Chemistry Unit Chief:

Date: 09/11/2019

**QA Approval**

Quality Manager:

Date: 09/11/2019

## **Guidelines for Comparison of Mass Spectra**

### **1 Scope**

This document provides guidelines to use when comparing mass spectra of known and unknown substances. This document does not address identification of unknown substances. Mass spectrometry is only one of many techniques that may be used in an attempt to identify an unknown substance. All acquired data will be considered when identifying an unknown substance. If structural elucidation is achieved with a technique other than mass spectrometry, then the calculation and comparison of ion ratios is not required.

These guidelines are intended for application to full scan, tandem, and selected ion monitoring (SIM) mass spectra acquired in electron impact (EI), chemical (CI), electrospray (ESI), and atmospheric pressure chemical (APCI) ionization modes. Other mass spectral techniques are beyond the scope of this document. Any specific mass spectral comparison guidelines in individual General Chemistry standard operating procedures (SOPs) will override any guidelines set forth in this document.

This document applies to Chemistry Unit (CU) personnel that are qualified to examine General Chemistry evidence.

### **2 Equipment/Materials/Reagents**

Not applicable

### **3 Standards and Controls**

Not applicable

### **4 Sampling**

Not applicable

## **5 Procedure**

### **5.1 Background-Subtracted Mass Spectrum**

Generate a background-subtracted mass spectrum. The background spectra may come before and/or after the peak of interest, and will all be selected from outside the region integrated for determination of ion ratios. This background-subtracted spectrum will be used to establish the base peak and significant ions.

### **5.2 Significant Ions**

Any ion signal greater than ~15% of the most intense ion signal in a background-subtracted mass spectrum will normally be considered a significant ion. An ion that would otherwise be considered significant may be excluded if it can be demonstrated that the ion arises from, or is significantly affected by a chemical interference. This interference can be demonstrated by showing that a reconstructed ion trace for the ion in question does not coincide with the traces for other ions associated with the peak of interest.

### **5.3 Diagnostic Ions**

Diagnostic ions in a mass spectrum are those ions that are characteristic of the chemical compound. Determination of diagnostic ions depends upon knowledge of the chemical structure and assessment of the mass spectrum of reference materials. There is not a universally accepted standard for determining diagnostic ions, however, the following recommendations will be considered.

- Adduct ions will normally be excluded, except that one pseudo-molecular adduct ion may be considered diagnostic.
- Isotopes will be excluded unless they are characteristic of a specific chemical composition (e.g., chlorine, bromine).
- Ions resulting purely from a derivatizing or complexing reagent will normally be excluded from the list of diagnostic ions.
- The molecular (or pseudo-molecular) ion will be considered diagnostic, unless the intensity for that ion is less than ~5%.

### **5.4 Base Peak**

The base peak for the mass spectrum of a reference material is the most intense signal for a diagnostic ion in the background-subtracted mass spectrum. For the purpose of calculating ion

ratios, the base peak in an unknown mass spectrum will be defined as the base peak of the reference material spectrum to which it is being compared.

## 5.5 Calculating Ion Ratios

Ion ratios will be determined by integrating reconstructed ion traces for the selected diagnostic ions. Integrations of reconstructed ion traces from a given spectrum will have comparable start and stop points. Ion ratios are then calculated by dividing the area of each ion trace by the area of the base peak ion trace, and expressing the result as a percentage. In instances where the reconstructed ion traces produce non-integratable data, it is acceptable to substitute ion abundances from the background subtracted spectra.

## 5.6 Decision Criteria

### 5.6.1 Full Scan Mass Spectra

- a. Every significant ion present in the known spectrum should be present in the unknown spectrum, and vice-versa.
- b. The ion ratios for diagnostic ions in the unknown spectrum should fall within the ranges provided in Table 1 or Table 2. If these limits would produce an acceptable lower bound of less than 1% for a given ion ratio, the lower limit will be set to 1%. Ion ratios for specific diagnostic ions may be excluded from consideration if they meet any of the following criteria:
  1. The ion ratio in the known spectrum is less than 5% (less than 10% for CI, ESI, or APCI spectra).
  2. It can be shown that the signal in either the known or the unknown spectrum is significantly disturbed by an uncorrectable chemical interference. Such interference will normally be demonstrated by showing that a reconstructed ion trace for the ion in question is not coincident with the traces for other ions associated with the component of interest.

If there are more than four diagnostic ions in the known spectrum, then only the ratios for four diagnostic ions (three ratios) need to be evaluated. For compounds with a molecular mass less than 80 Da, or consisting of less than 8 atoms, only three diagnostic ions (2 ratios) need to be evaluated. The selected ions will normally include the base peak and the molecular (or pseudo-molecular) ion, unless those ions meet one of the exclusion criteria given above. If fewer than three diagnostic ions are available for evaluation, the spectra may still be compared, but information derived from such a comparison is limited.

Table 1: Ion Ratio Ranges for EI Mass Spectra

If the ion ratio in the known spectrum is:	>50%	≥25% and ≤50%	<25%
Then the ion ratio in the unknown spectrum should be within:	10% absolute	20% relative	5% absolute

Table 2: Ion Ratio Ranges for CI, ESI, and APCI Mass Spectra

If the ion ratio in the known spectrum is:	>60%	≥40% and ≤60%	<40%
Then the ion ratio in the unknown spectrum should be within:	15% absolute	25% relative	10% absolute

## 5.6.2 SIM Mass Spectra

Four diagnostic and significant ions (if available) will normally be selected when setting up a SIM experiment (three ions for compounds with a molecular mass less than 80 Da or consisting of less than 8 atoms). The base peak will normally be one of the chosen ions, and the molecular (or pseudo-molecular) ion will be included if it has an ion ratio greater than ~5% in the known full scan spectrum. The ion ratios for diagnostic ions in the unknown spectrum should fall within the ranges provided in Table 1 or Table 2.

## 5.6.3 Tandem Mass Spectrometry (MS/MS)

The limit for determination of significant ions in a tandem mass spectrum is ~10% of the most intense observed ion in the background subtracted spectrum. The high probability of ion association in tandem mass spectrometry means that nearly all ions of reasonable intensity observed in an MS/MS experiment should be considered diagnostic, with the exception of ions resulting purely from the loss of an adduct.

Due to the physical processes involved in precursor ion isolation and fragmentation events in an ion trap mass spectrometer, tandem mass spectra acquired on such an instrument will occasionally show an ion-splitting artifact for a precursor ion returned in a product ion mass spectrum. This is evidenced by the presence of two ions, separated by a fraction of  $m/z$ , at the nominal  $m/z$  of the precursor ion in the product ion spectrum. In instances where this phenomenon is observed, the response for the affected ion will be taken as the total of the response for both components of the split ion signal.



### 5.6.3.1 Product Ion Experiments

When conducting product ion experiments, the selection of a precursor ion is critical to obtaining useful and reliable information. In most cases, the molecular (or pseudo-molecular) ion of the species under consideration will be selected, if available. It is also acceptable to use a diagnostic isotope of the molecular (or pseudo-molecular) ion, if one is available. If the molecular (or pseudo-molecular) ion is not available, or is not suitable for some reason, then the selected precursor ion should be both significant and diagnostic in the full scan mass spectrum of the substance under consideration. With product ion spectra, it is also important to ensure that the observed fragment spectrum is emerging from the selected precursor ion. For this reason, one of the two following criteria should normally be met for a product ion spectrum:

- a. The precursor ion should be observed in the product ion spectrum with an ion ratio of at least 5%.
- b. If full scan mass spectral data are collected concurrently with the product ion spectra, the full scan spectrum of the component of interest should show no ions within 1.5  $m/z$  of the precursor ion with greater than three times the intensity of the precursor ion.

Following are decision criteria when comparing product ion spectra:

- a. Every significant ion present in the known spectrum should be present in the unknown spectrum, and vice-versa.
- b. The ion ratios for diagnostic ions in the unknown spectrum should fall within the ranges provided in Table 3. If these limits would produce an acceptable lower bound of less than 1% for a given ion ratio, the lower limit will be set at 0.5%. Ion ratios for specific diagnostic ions may be excluded from consideration if they meet any of the following criteria:
  1. The ion ratio for that ion in the known spectrum is less than 5%.
  2. It can be shown that the signal in either the known or the unknown spectrum is significantly disturbed by an uncorrectable chemical interference. Such interference will normally be demonstrated by showing that a reconstructed ion trace for the ion in question is not coincident with the traces for other ions associated with the component of interest.

If there are more than three diagnostic ions in the known spectrum, then only the ratios for three diagnostic ions (two ratios) need to be evaluated. The three selected ions will include the base peak and the precursor ion (if present), unless those ions meet one of the exclusion criteria given above. If only a single diagnostic ion is observed in the product ion spectrum, spectra may still be compared, but information derived from such a comparison is limited.

Table 3: Ion Ratio Ranges for MS/MS Product Ion Spectra

If the ion ratio in the known spectrum is:	>40%	≤40%
Then the ion ratio in the unknown spectrum should be within:	25% relative	10% absolute

### 5.6.3.2 Precursor Ion and Neutral Loss Experiments

The practical information content for precursor ion and neutral loss MS/MS experiments is generally low, but circumstances may still arise in which one of these techniques can provide critical additional information about a given substance. For precursor ion experiments all significant ions present in the known spectrum should be present in the unknown spectrum, and vice-versa. For neutral loss experiments, all significant transition pairs present in the known spectrum should be present in the unknown spectrum and vice-versa.

### 5.6.3.3 Selected Reaction Monitoring (SRM) Experiments

Two or three diagnostic ion transitions may be chosen for an SRM experiment. Generally, transitions should share a common precursor ion, although it is appropriate to use multiple precursor ions if all are part of a diagnostic isotope cluster in the full scan spectrum of the substance in question. It is desirable that the chosen precursor ion be the molecular (or pseudo-molecular) ion of the substance in question. If this is not possible, or not practical, then the chosen precursor ion should be both significant and diagnostic in the full scan spectrum of the substance in question. When comparing a known SRM spectrum and an unknown SRM spectrum, the ion ratio of the unknown should be within  $\pm 10\%$  (relative) of the ion ratio of the known when only two transitions are monitored. When three transitions are monitored, both resulting ion ratios should meet the tolerances specified in Table 3.

### 5.6.4 Accurate Mass Spectrometry

Ions in an unknown measured accurate mass spectrum should be within  $0.005\ m/z$  of the ions in the known measured accurate mass spectrum, or the theoretical  $m/z$  (i.e., exact mass). Any isotope of a molecular (or pseudo-molecular) ion may be considered diagnostic if it meets the  $0.005\ m/z$  criterion. One additional adduct ion, beyond the pseudo-molecular ion, may also be considered diagnostic if it meets the  $0.005\ m/z$  criterion. For a SIM experiment, only three ions need to be evaluated if all three ions meet the  $0.005\ m/z$  criterion.

### 5.6.5 Library Spectra Comparisons

While mass spectral libraries can be invaluable tools in helping to direct examinations and suggest possible targets for further investigation, there are limitations to their use. Most

commercial libraries do not clearly indicate the instrumentation the spectra were acquired on, or at what level of sample loading. In-house library data may have been acquired on the same instrumentation used to obtain a given unknown spectrum, but it is very difficult to ensure that long-term drift in instrument performance has not affected the utility of those library spectra.

Despite these limitations, there may arise instances in which it is necessary to compare an unknown spectrum to a library entry. When such comparisons are conducted, all criteria described above will be utilized. However, ion abundances for the determination of ion ratios will be measured as the intensity of the ion in the spectrum, rather than as the integrated area of a reconstructed ion trace.

## 6 Calculations

$IR_x = (A_x/A_b) \times 100$ , where:

$IR_x$  = the percent ion ratio for ion x

$A_x$  = the integrated area of the reconstructed ion trace for ion x

$A_b$  = the integrated area of the reconstructed ion trace for the base peak ion

(Ion abundances from background subtracted mass spectra may be substituted for integrated areas under certain circumstances detailed in section 5.6.5.)

## 7 Measurement Uncertainty

Not applicable

## 8 Limitations

These guidelines are not intended to be exhaustive. Known limitations for specific analytes will be documented in the applicable SOPs.

## 9 Safety

Not applicable

## 10 References

Betham R, Boison J, et al. Estimating the fitness for purpose of mass spectrometric methods, *J Am Soc Mass Spectrom* 2003; 14: 528-541

deZeeuw, RA. Letter to the Editor – Fitness for purpose of mass spectrometric methods in substance identification, *J Forensic Sci* 2005; 50(3): 745-747

McLafferty FW, Stauffer DA, Loh SY, Wesdemiotis C. Unknown identification using reference mass spectra. Quality evaluation of databases. *J Am Soc Mass Spectrom* 1999; 10: 1229-1240

Kidwell DA, Riggs LA. Comparing two analytical methods: minimal standards in forensic toxicology derived from information theory, *Forensic Sci Int* 2004; 145: 85-96

deZeeuw RA. Substance identification: the weak link in analytical toxicology, *J Chrom B* 2004; 811: 3-12

World Anti-Doping Agency. Technical Document TD2015IDCR: Minimum criteria for chromatographic-mass spectrometric confirmation of the identity of analytes for doping control purposes. Montreal, Canada: 2015

US Department of Health and Human Services / Food and Drug Administration Center for Veterinary Medicine. Guidance Document 118: Mass spectrometry for confirmation of the identity of animal drug residues. Rockville MD: 2003

Rev. #	Issue Date	History
0	01/15/20	Original Issue

**Approval**

Redacted - Signatures on File

Chemistry Unit Chief:

Date: 01/14/2020

General Chemistry  
Technical Leader:

Date: 01/14/2020

## **General Chemistry Instrument Parameters**

### **1 Scope**

This document provides parameters for commonly used instruments in General Chemistry analyses and is to be used in conjunction with General Chemistry standard operating procedures (SOPs) and Chemistry Unit (CU) instrument Performance Monitoring Protocols. Any specific instrument parameters or decision criteria in individual General Chemistry SOPs will override those set forth in this document.

This document applies to CU personnel that are qualified to examine General Chemistry evidence.

### **2 Equipment/Materials/Reagents**

- Fourier Transform Infrared (FTIR) spectrophotometer with Attenuated Total Reflectance (ATR), transmission, or microscope attachments
- Raman spectrophotometer with microscope and/or bench capabilities
- 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)
- GC/MS equipped with a chemical ionization source and a 30 meter DB-5 column (or equivalent)
- GC/MS equipped an electron impact ionization source and a headspace autosampler (HS-GC/MS) and a 30 meter DB-624 column (or equivalent)
- Gas chromatograph equipped with a flame ionization detector (GC-FID), a headspace autosampler (HS-GC-FID) and a 30 meter Restek Rtx-BAC-2 column (or equivalent)
- Gas chromatograph equipped with a nitrogen/phosphorus detector (GC-NPD), a headspace autosampler (HS-GC-NPD) and a 30 meter Restek Rt-QS-Bond column (or equivalent)
- GC-NPD equipped with a 30 meter Rtx-1701 column (or equivalent)
- Gas chromatograph equipped with an electron capture detector (GC-ECD) and a 30 meter Rtx-CLPest column (or equivalent)
- High-temperature GC-FID equipped with a 15 meter Zebron “Inferno” ZB-1HT column (or equivalent)
- Liquid chromatography system with appropriate column coupled to a mass spectrometer (LC/MS) with electrospray ionization (ESI) or atmospheric pressure

chemical ionization (APCI) (e.g., Thermo LTQ, Thermo LTQ OrbiTrap XL, Thermo Exactive Orbitrap)

- Ion chromatograph for anions
- Ion chromatograph for cations
- Scanning electron microscope with energy dispersive X-ray spectrometer (SEM/EDS)
- X-ray powder diffractometer (XRD)
- Thermo QUANT'X X-ray Fluorescence Spectrometer (XRF)
- Bruker M4 Tornado Micro X-ray Fluorescence Spectrometer (micro-XRF)
- Ultraviolet-Visible spectrophotometer (UV-Vis)

### 3 Standards and Controls

Refer to the applicable General Chemistry SOPs, Metallurgy SOPs (for XRF), and/or instrument Performance Monitoring Protocols for the preparation of all standards and/or controls.

### 4 Sampling

Not applicable

### 5 Procedure

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 (or *similar* conditions for some techniques, e.g. SEM/EDS) 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.

#### 5.1 FTIR

	ATR	Transmission	Microscope
<b>No. of scans</b>	32	32	128
<b>Resolution</b>	4	4	4
<b>Final format</b>	% Reflectance	% Transmittance	% Transmittance
<b>Correction</b>	None	None	None

## 5.2 Raman

	Microscope and Bench
Collect exposure time (sec)	10.0
Preview exposure time (sec)	1.0
Sample exposures	4
Background exposures	4
Final format	Shifted spectrum (cm <sup>-1</sup> )
Correction	White light
Cosmic ray threshold	Low

## 5.3 UV/Vis

Wavelength scan mode	
Start wavelength	800 nm
End wavelength	220 nm
Integration time	0.25 sec
Data interval	0.5 nm
Scan speed	120 nm/min

## 5.4 SEM/EDS

SEM Settings (record the following SEM parameters)	
Accelerating voltage	(typically 25.0 kV)
Imaging mode	(SEI or BEI, at minimum)
Magnification	
Working distance	(~13 mm ideal for EDS)
Count rate and/or dead time	(~4000 cps ideal for EDS)
EDS Settings	
Preset	200.0 Live
Amp time	Auto

## 5.5 XRF

Refer to the following Metallurgy SOPs for XRF parameters:

*Compositional Analysis by Energy Dispersive X-Ray Fluorescence Spectrometry (CU Metallurgy 500)*

*Operation of the Thermo QUANT'X X-Ray Fluorescence Spectrometer (CU Metallurgy 501)*



*Operation of the Bruker M4 Tornado X-Ray Fluorescence Spectrometer (CU Metallurgy 502)*

## 5.6 XRD

	<b>Miniflex II</b>	<b>Miniflex 600</b>
<b>X-ray source</b>	30 kV, 15 mA	40 kV, 15 mA
<b>Scan mode</b>	Continuous	Continuous
<b>Scan speed</b>	40 deg/min	40 deg/min
<b>Step width</b>	0.02 deg	0.02 deg
<b>Scan range</b>	5-75 deg	5-75 deg

## 5.7 GC/MS (EI)

### 5.7.1 Electron Impact (EI)

<b>GC Settings</b>	
Injection mode	Split or Splitless
Injection volume	1 uL
Inlet temperature	250 °C
Oven program	60 °C for 2 min, 35 °C /min to 260 °C for 15 min
<b>Full Scan MS Settings</b>	
Polarity	Positive
Solvent delay	3.00 min
Scan range	43 to 400 <i>m/z</i>

### 5.7.2 Chemical Ionization (CI)

<b>GC Settings</b>	
Injection mode	Split or Splitless
Injection volume	1 uL
Inlet temperature	250 °C
Oven program	60 °C for 2 min, 35 °C /min to 260 °C for 15 min
<b>Full Scan MS Settings</b>	
Polarity	Positive or Negative
Solvent delay	3.00 min
Reagent gas	Methane
Scan range	100 to 400 <i>m/z</i>

## 5.8 GC-ECD

GC Settings	
Injection mode	Splitless
Injection volume	1 uL
Inlet temperature	230 °C
Oven program	125 °C for 1 min, 7 °C /min to 280 °C for 22 min
ECD Settings	
Temperature	300 °C
Makeup gas	Nitrogen
Makeup flow	30 mL/min

## 5.9 GC-NPD

GC Settings	
Injection mode	Split
Split ratio	15:1
Injection volume	1 uL
Inlet temperature	250 °C
Oven program	125 °C for 1 min, 7 °C /min to 280 °C for 22 min
NPD Settings	
Temperature	250 °C
Offset	10
Makeup gas	Nitrogen
Makeup flow	30 mL/min
Air flow	60 mL/min
Hydrogen flow	2 mL/min

## 5.10 High Temperature GC-FID

Oven program	55 °C for 2 min, 30 °C /min to 100 °C for 0 min, 15 °C /min to 400 °C for 3.5 min
<b>PTV Inlet</b>	
Injection mode	Splitless
Injection volume	1 uL
Initial temperature	55 °C
Ramp	500 °C to 400 °C for 10 min
Pressure	7 psi
Total flow	34 mL/min
<b>FID Settings</b>	
Temperature	420 °C
Mode	Constant makeup flow
Hydrogen flow	40.0 mL/min
Air flow	450.0 mL/min
Makeup flow	30.0 mL/min

## 5.11 Headspace GC/MS (EI)

<b>GC Settings</b>	
Injection mode	Split
Split ratio	10:1
Inlet temperature	150 °C
Oven program	50 °C for 3 min, 10 °C /min to 250 °C for 21.5 min
<b>Headspace Autosampler Settings (Maestro)</b>	
Incubation time	30 min
Incubation temperature	80 °C
Syringe	2.5 mL-HS
Syringe temperature	90 °C
Injection volume	1000 uL
<b>Full Scan MS Settings</b>	
Polarity	Positive
Solvent delay	1.75 min
Scan range	27 to 400 <i>m/z</i>

## 5.12 Headspace GC-FID

<b>GC Settings</b>	
Injection mode	Splitless
Split ratio	10:1
Oven program	50 °C for 3 min, 10 °C /min to 250 °C for 21.5 min
<b>Headspace Autosampler Settings (Maestro)</b>	
Incubation time	30 min
Incubation temperature	80 °C
Syringe	2.5 mL-HS
Syringe temperature	90 °C
Injection volume	1000 uL
<b>FID Settings</b>	
Heater temperature	250 °C
H <sub>2</sub> flow	40 mL/min
Air flow	400 mL/min

## 5.13 Headspace GC-NPD

<b>GC Settings</b>	
Back PP Inlet N2	
Inlet temperature	150 °C
Oven program	110 °C for 0 min, 4 °C /min to 130 °C for 5 min
<b>Headspace Autosampler Settings (Maestro)</b>	
Incubation time	5 min
Incubation temperature	45 °C
Syringe	2.5 mL-HS
Syringe temperature	55 °C
Injection volume	250 uL
<b>NPD Settings</b>	
Maximum bead voltage	4.095 V
Dry bead	Yes
Heater	225 °C

## 5.14 LC/MS

<b>LC Settings</b>	
Column dimensions	Length 150 mm, Diameter 2.1 mm
Particle size	5 $\mu\text{m}$
Column oven temperature	30 °C
Flow rate	0.3 mL/min
<b>MS Settings</b>	
Ionization mode	ESI
Sheath gas	20
Aux gas	10
Sweep gas	5
Capillary temperature	275 °C
Scan mode	Full
Scan range	100-650 $m/z$
<b>High Resolution MS Settings</b>	
Resolving power	30,000

## 5.15 DART/TOFMS

<b>DART Source Settings</b>	
Polarity	Positive or Negative
Source gas	Helium
Temperature	400 °C

<b>TOFMS Settings</b>	
Polarity	Same as DART Source
Ion guide RF (or equivalent)	800 V for 80-800 $m/z$ scan range, 500 V for 50-500 $m/z$ scan range

## 5.16 Ion Chromatography

### 5.16.1 Cations

	<b>Waters</b>	<b>Dionex</b>
Mobile phase	3.0 mM HNO <sub>3</sub> /0.1 mM EDTA	20 mM methanesulfonic acid
Pump mode	Isocratic	Isocratic
Flow rate	1.0 mL/min	1.0 mL/min
Column	Waters IC-Pak C M/D	Dionex IonPac CS12A
Column temperature	Ambient	30 °C
Injection volume	10 uL	25 uL
Acquire time	15 min	15 min
Detector	Waters 432 conductivity detector	Suppressed conductivity detector

### 5.16.2 Anions

	<b>Dionex (KOH)</b>	<b>Dionex (K<sub>2</sub>CO<sub>3</sub>)</b>
Mobile phase	20 to 80 mM potassium hydroxide	10 mM potassium carbonate
Pump mode	Multi-step gradient 20 mM at 0 min 20 mM at 2 min 30 mM at 9 min 80 mM at 13 min 80 mM at 21 min 20 mM at 21.1 min 20 mM at 25 min	Isocratic
Flow rate	1.0 mL/min	1.5 mL/min
Column	Dionex IonPac AS19 with IonPac AG19 guard	Dionex IonPac AS22 with IonPac AG22 guard
Column temperature	30 °C	30 °C
Injection volume	25 uL	20 uL
Acquire time	25 min	16 min
Detector	Suppressed conductivity detector	Suppressed conductivity detector

## 6 Calculations

Not applicable

## 7 Measurement Uncertainty

Not applicable

## 8 Limitations

The following criteria are used as guidelines in determining the acceptability of data. Any specific criteria in individual General Chemistry SOPs will override the below criteria.

### 8.1 Chromatography

- Peaks should show good chromatographic characteristics, with reasonable peak shape, width, and resolution.
- The retention time of a peak should be within  $\pm 2\%$  for GC and  $\pm 5\%$  for LC when compared to a contemporaneously analyzed reference standard or Positive Control.
- The signal intensity for a peak should be at least 10x greater than the intensity from any carryover peak which is present in the preceding blank or the Negative Control.

### 8.2 Mass Spectrometry

- The mass spectrum of the analyte of interest should compare favorably with that of a contemporaneously analyzed reference standard or Positive Control. See *Guidelines for Comparison of Mass Spectra* (GenChem 33) for further guidance.
- DART/TOFMS peaks of interest should be within  $\pm 0.005 m/z$  of a contemporaneously analyzed reference standard or Positive Control, and/or the theoretical accurate mass value of the ion of interest.

### 8.3 SEM/EDS and XRF

The instrument software and KLM reference lines are used to label any peaks that are present in a collected spectrum using the following systematic approach:

- Label any high-energy peaks and major peaks. High-energy peaks are less likely to have overlapping peaks when compared to low-energy peaks. If a major peak is present, then a complete family of peaks can generally be identified.
- Major peaks may give rise to spectral artifacts, such as sum peaks and escape peaks. Check the spectrum for the presence of these artifacts and label any that are identified. Keep in mind that sum peaks may arise from more than 1 element if multiple major peaks are present.
- Attempt to label any remaining, lower intensity peaks. This may require adjustment of the vertical scale to reveal the necessary detail. Inconsistent peak ratios (when

compared to reference lines) or asymmetric peaks may indicate peak overlap. Weak intensities and/or peak overlap may prevent identification of an element within the spectrum.

- The presence of an element may be considered unequivocal only when a characteristic, unique set of lines is produced, or when a single peak occurs at an energy where it cannot be mistaken for another element or a spectral artifact. Unequivocal identification may not be possible if an element is present at a low concentration and/or if its lines overlap with signals from other elements present in the sample.
- Unequivocal elemental assignments are represented by labeling the peak with the elemental symbol.
- Probable (i.e., not unequivocal) elemental assignments are represented by labeling the peak with parentheses around the elemental symbol.
- Escape peaks may be represented by the addition of “- Si” to the elemental symbol (e.g. Cl – Si), or simply by annotating as “Escape” or similar.
- Sum peaks are represented by the “+” sign (e.g., C + O).
- Possible peaks that are too weak to assess are labeled as “?” or similar.
- Additional information may be included with peak labels (e.g., series and shell).

#### 8.4 All Other Data

- Data from the analyte of interest should compare favorably with that of a contemporaneously analyzed reference standard, Positive Control, and/or library entry.
- The signal intensity for a peak should be at least 10x greater than the intensity of a corresponding peak which is present in a preceding blank or the Negative Control.

### 9 Safety

Take standard precautions for the handling of all chemicals, reagents, and reference 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. Personnel operating an XRF or XRD instrument will wear a dosimeter badge.



## **10 References**

### **10.1 Instrument Operation & Systems Support Protocols**

*Performance Monitoring Protocol (QA/QC) for the Agilent Headspace GC/MS*

*Performance Monitoring Protocol (QA/QC) for the Agilent 7890/5975 Headspace GC/FID/MS*

*Performance Monitoring Protocol (QA/QC) for the Agilent GC/MS*

*Performance Monitoring Protocol (QA/QC) for the JEOL AccuTOF DART*

*Performance Monitoring Protocol (QA/QC) for the Thermo LTQ LC/MS (ESI)*

*Performance Monitoring Protocol (QA/QC) for the Thermo LTQ OrbiTrap XL LC/MS (ESI)*

*Performance Monitoring Protocol (QA/QC) for the Thermo Exactive OrbiTrap LC/MS (ESI)*

*Performance Monitoring Protocol (QA/QC) for the Agilent 7890 GC/ECD/NPD*

*Performance Monitoring Protocol (QA/QC) for the Agilent 7890 High Temperature GC/FID*

*Performance Monitoring Protocol (QA/QC) for the Ion Chromatography (IC) System*

*Performance Monitoring Protocol (QA/QC) for the Thermo Nicolet FTIRs*

*Performance Monitoring Protocol (QA/QC) for the UV-Vis Spectrophotometer*

*Performance Monitoring Protocol (QA/QC) for the Raman Spectrometers*

*Performance Monitoring Protocol (QA/QC) for the Scanning Electron Microscope (SEM) /  
Energy Dispersive X-ray Spectrometer (EDS)*

*Performance Monitoring Protocol (QA/QC) for the Rigaku Miniflex X-ray Diffractometer (XRD)*

### **10.2 Metallurgy SOPs**

*Compositional Analysis by Energy Dispersive X-Ray Fluorescence Spectrometry*

*Operation of the Thermo QUANT'X X-Ray Fluorescence Spectrometer*

*Operation of the Bruker M4 Tornado X-Ray Fluorescence Spectrometer*

### **10.3 Additional References**

*FBI Laboratory Safety Manual*

*Analysis of General Unknowns*; FBI Laboratory Chemistry Unit - General Chemistry SOP  
(GenChem 1)

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

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**Approval**

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Chemistry Unit Chief:

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General Chemistry  
Technical Leader:

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