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 and authorized to examine General Chemistry 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
- Chloroform
- Deionized water
- 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 within 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.

A known prescription preparation may also be used to prepare a Positive Control.

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 on a heterogeneous item, the results of examinations will be clearly limited to the sample(s) that were selected and examined.

6 Procedure

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

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. Test tubes and other containers may be used in place of spot plates. The reagent and sample

amounts may be adjusted as necessary. 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 (or edit the current 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 (or edit the current 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 (see Appendix A).

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).

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

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

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

$$X\ mg\ tablet = 10.1\ mg$$

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 electronically in CU.

9 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*.

The following conclusions apply to the analysis of drugs:

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

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

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

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

10 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. Some of the chemicals may be carcinogenic. Refer to the *FBI Laboratory Safety Manual* for the proper handling and disposal of all chemicals. Personal protective equipment should be used when handling any chemical and when performing any type of analysis.

11 References

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*, 8th Edition, 2019

Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), *SWGDRUG Monographs*, 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

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

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

Drug Analysis; FBI Laboratory Chemistry Unit – General Chemistry SOP (GenChem 6)

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

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

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

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

Chemistry Unit Validation of Analytical Procedures; FBI Laboratory Chemistry Unit – Quality Assurance and Operations Manual (CU QAOM 11)

FBI Laboratory Safety Manual

Rev. #	Issue Date	History
2	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.
3	04/01/21	Section 1- added "and authorized" and edited to include only General Chemistry evidence. Section 2- removed ammonium hydroxide and diethyl ether. Section 3.2- minor grammatical edit ("into" to "within"); added last sentence. Section 5- added "on a heterogeneous item". Section 6- added first sentence; step (d)- allowed for test tubes and other containers, as well as changes to reagent and sample amounts; steps (i) and (j)- added "(or edit the current sequence)"; step (j)- added reference to Appendix A. Deleted previous sections 9 and 10. Section 8- edited last sentence to remove "s:\ drive". Section 9- added the content below the first bulleted list. Section 11- added GenChem 34; added parenthetical references to end of CU SOPs.

Approval

Redacted - Signatures on File

Chemistry Unit Chief: Date: 03/31/2021

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

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_2 (g) flow at ~60 ºC.

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_2 (g) flow at ~60 ºC.