Drug Analysis

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Drug Analysis

1 INTRODUCTION

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, drug paraphernalia (e.g., smoking devices, syringes), prescription medications, over-the-counter products, vegetative substances, and paper substrates. 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. Results are compared to acquired reference materials (when available) that are analyzed on the same equipment under the same conditions.

While this procedure may be used to identify a vegetative substance as cannabis, the procedure does not allow for the identification of the substance as marijuana or hemp.

2 SCOPE

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

3 EQUIPMENT

- General laboratory supplies
- Aerosol sprayer
- Analytical balance
- Evaporator
- Digital microscope
- Stereo microscope
- Ultraviolet light source (long wavelength)
- Acetaldehyde
- Acetonitrile
- Chloroform
- Cobalt thiocyanate
- Deionized water
- Diethyl ether
- Ethanol
- Fast Blue BB Salt
- Formaldehyde (40%)
- Formic Acid
- Hydrochloric acid
- Methanol (MeOH)
- Nitric acid
- Petroleum ether
- Sodium bicarbonate
- Sodium carbonate
- Sodium nitroprusside (aka sodium nitroferricyanide)
- Sodium sulfate (anhydrous)

- Sulfuric acid
- Toluene
- Silica gel Thin-Layer Chromatography (TLC) plate and TLC tank
- 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)
- Liquid chromatography system with Waters Xterra C18 MS column (or equivalent) coupled to a mass spectrometer (LC/MS) with electrospray ionization (ESI) (e.g., Thermo LTQ, Thermo LTQ OrbiTrap XL, Thermo Exactive OrbiTrap)

4 STANDARDS AND CONTROLS

4.1 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.

4.2 Positive Control

4.2.1 Prepared from Solids

Prepared by making a 1 mg/mL (as base) stock standard solution of the applicable drug within a suitable solvent. The desired working concentration is then prepared by diluting the stock standard solution (e.g., a 0.1 mg/mL solution is prepared by diluting the stock standard solution 1:10). These solutions will be stored in a freezer or refrigerator and are verified with each use. Other concentrations may be prepared and used as needed.

4.2.2 Prepared from Liquids

Prepared by diluting a reference material solution (e.g., a 0.1 mg/mL solution is prepared by diluting a 1 mg/mL reference material solution 1:10 in an appropriate solvent). These solutions will be stored in a freezer or refrigerator and are verified with each use. Other concentrations may be prepared and used as needed.

4.2.3 Medications

A known medication preparation (e.g., prescription, over-the-counter) may also be used to prepare a Positive Control in a similar manner as described above depending on the nature of the medication preparation.

5 PREPARATION OF COLOR TEST REAGENTS

5.1 Marquis Reagent

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

5.2 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 an amber 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.

5.3 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 at room temperature.
- 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.

5.4 Hydrochloric Acid [0.1 N (aq)]

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

5.5 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.

6 SAMPLING

Statistical sampling is performed according to GENCHEM-301.

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.

7 PROCEDURE

Appendix A contains a general drug analysis flow chart.

Refer to GENCHEM-302 for specific instrument settings and decision criteria.

7.1 Visual Examination

Perform a visual examination of each item. Microscopy may be used as deemed necessary. See Table 1 for guidance.

Table 1- Visual Examination Guidance

Specimen Type	Guidance
'Bulk' substance (i.e., solid, liquid)	• Observe and record general physical properties, to include if specimen appears homogeneous/heterogeneous
Paraphernalia (e.g., smoking devices, syringes, containers, straws, grinders)	• Observe items for potential to directly sample residual substance • If a solvent rinse will be necessary, consider impact on other forensic examinations (e.g., DNA, latent fingerprints)
Tablets, pills, capsules, etc.	• Record total tablet count and relevant physical characteristics (e.g., size, shape, color, imprints/logos, score marks) • Search physical characteristics with a resource such as Drugs.com Pill Identifier or the DEA Logo Index for Tablets and Capsules
Potentially soaked matrices (e.g., letters, envelopes, pamphlets)	• Observe for signs of exposure to liquids • Utilize alternate light sources as necessary • Photograph significant observations
Intact vegetative substance (possible Cannabis)	• Cannabis is typically green, brown, or a variation between the two • Observe with microscope for cystolithic ("bear claw" shaped) and glandular (also referred to as trichomes) hairs (glandular hairs often contain beads of resin on the ends) • The odor, or any other significant characteristics of the item, should be noted
Lysergic Acid Diethylamide (LSD)	• Observe under long wavelength ultraviolet light for blue fluorescence

7.2 Weight

Use a traceable analytical balance to record the weight for each item, as relevant. For example, the weight of a drug paraphernalia item prior to rinsing is not typically relevant.

7.3 Sample Preparation

Prior to analysis by one or more of the techniques in Sections 7.4 through 7.9, a sample may be prepared from an item by one or more of the following methods. The techniques in Sections 7.4 through 7.9 may also be performed directly on a portion of an item (i.e., neat).

7.3.1 Extraction/Dilution

An appropriate amount of the item may be placed into a small test tube and extracted/dissolved/diluted in an appropriate solvent (e.g., methanol, chloroform, acetonitrile) to achieve the desired concentration. For example, an active ingredient concentration of ~1 mg/mL may be desirable for DART/TOFMS analysis, while ~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 B for acid/neutral and alkaline drug extraction steps.

7.3.2 Rinse

Paraphernalia items that do not have recoverable material may be rinsed. For example, rinse the interior of a pipe or an empty syringe barrel with ~1 mL of an appropriate solvent (e.g., methanol, chloroform, acetonitrile).

- If a syringe has a needle attached and the item needs to be preserved for future DNA exams, then avoid exposing the needle to solvent.
- If necessary, filter the rinse or centrifuge and decant to remove any undissolved particulates. The Negative Control will be filtered or centrifuged as well.
- Transfer the rinse to a labeled test tube, and if necessary, concentrate the rinse and Negative Control under N_2 (g) at ~60 °C.

7.3.3 Mixtures

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.

A. Homogenize a representative portion of the item with a mortar and pestle.

B. 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.

C. 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.

D. 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.

E. 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.

F. Isolate any remaining solids in the filter paper, as well as any solids recovered from the evaporated extracts.

7.4 Color Tests

Color tests may be performed, but are not required. 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 or 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.

7.4.1 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.

7.4.2 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.

7.4.3 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.

7.4.4 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.

7.5 FTIR-ATR

A representative sample may be analyzed by FTIR-ATR. The FTIR microscope attachment may be used as appropriate.

- A solid, powder, or pill/tablet item may be homogenized using a mortar and pestle.
    - If possible, homogenize approximately one-half of a pill/tablet, retaining the other half intact.
- A capsule may be opened to remove solid contents, or a syringe and needle may be used to remove liquid contents from a sealed capsule.
- Liquid samples may be analyzed neat and/or allowed to evaporate on the ATR cell.

7.6 DART/TOFMS

A representative sample 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.

7.7 Thin-Layer Chromatography (TLC)

As an alternative to DART/TOFMS, extracts suspected of containing delta-9-tetrahydrocannabinol (Δ^9-THC) may be analyzed by TLC.

A. 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.

B. Spot 5 to 10 uL of the extract(s) at the origin of a silica gel TLC plate (typically ≥ 1 cm from bottom of TLC plate). Spot 5 to 10 uL of the Δ^9-THC Positive Control and the extracts on the same TLC plate. Allow the spots to dry.

C. Place the TLC plate into the TLC tank and allow the mobile phase to migrate ~ 10 cm up the plate.

D. Remove the TLC plate from the tank, mark the location of the mobile phase solvent front, and allow the plate to dry.

E. Develop the plate by applying the Fast Blue BB solution via an aerosol sprayer. Red spots indicate the possible presence of cannabinoids.

F. Record and/or photograph the results. Calculate the retardation factor (R_f) for any red spots that are observed.

7.8 Gas Chromatography/Mass Spectrometry

7.8.1 Electron Impact (EI)

A solution prepared from an item 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).

7.8.2 Chemical Ionization (CI)

A solution prepared from an item 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 B).

7.9 Additional Techniques

Other analytical techniques not listed above may be used as deemed necessary provided 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 do not 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 CHEM-100. Examples of additional techniques include:

- 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)

8 CALCULATIONS

8.1 Tablet Example

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).

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

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

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

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

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

8.2 Thin-Layer Chromatography (TLC)

R_f = (distance spot center traveled) / (distance solvent front traveled)

Distances traveled are measured from the origin. Distances are considered approximate and R_f values are not treated as a significant measurement.

9 INSTRUMENTAL CONDITIONS

Refer to GENCHEM-302 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.

9.1 Liquid Chromatography/Mass Spectrometry (LC/MS)

The following conditions may be useful for the analysis of some synthetic cannabinoids.

9.1.1 Liquid Chromatography Parameters

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

9.1.2 Mass Spectrometer Parameters

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

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

9.1.2.2 Tandem Mass Spectrometry (MS/MS)

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

10 MEASUREMENT UNCERTAINTY

When a quantitative result (e.g., weight) is included in a *Laboratory Report*, measurement uncertainty will be estimated and reported (see CHEM-100). Uncertainty budget worksheets for each analytical balance approved for significant measurements are maintained electronically in CU.

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 technical procedure. If relevant isomeric forms of a compound are not differentiated, this will be clearly stated in the *Laboratory Report*.
- Testing of an item for identification of cannabis as hemp or marijuana (or as derived from hemp or marijuana) is not conducted in CU.

The following conclusions apply to the analysis of drugs:

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

Refer to GENCHEM-903, GENCHEM-201, 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 GENCHEM-302 for instrumental limitations and decision criteria.

Refer to GENCHEM-303 for mass spectra comparison decision criteria.

12 SAFETY

Fast Blue BB salt is a known carcinogen, handle with appropriate personal protective equipment.

13 REFERENCES

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

Drugs.com Pill Identifier, www.drugs.com/pill_identification.html

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

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

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

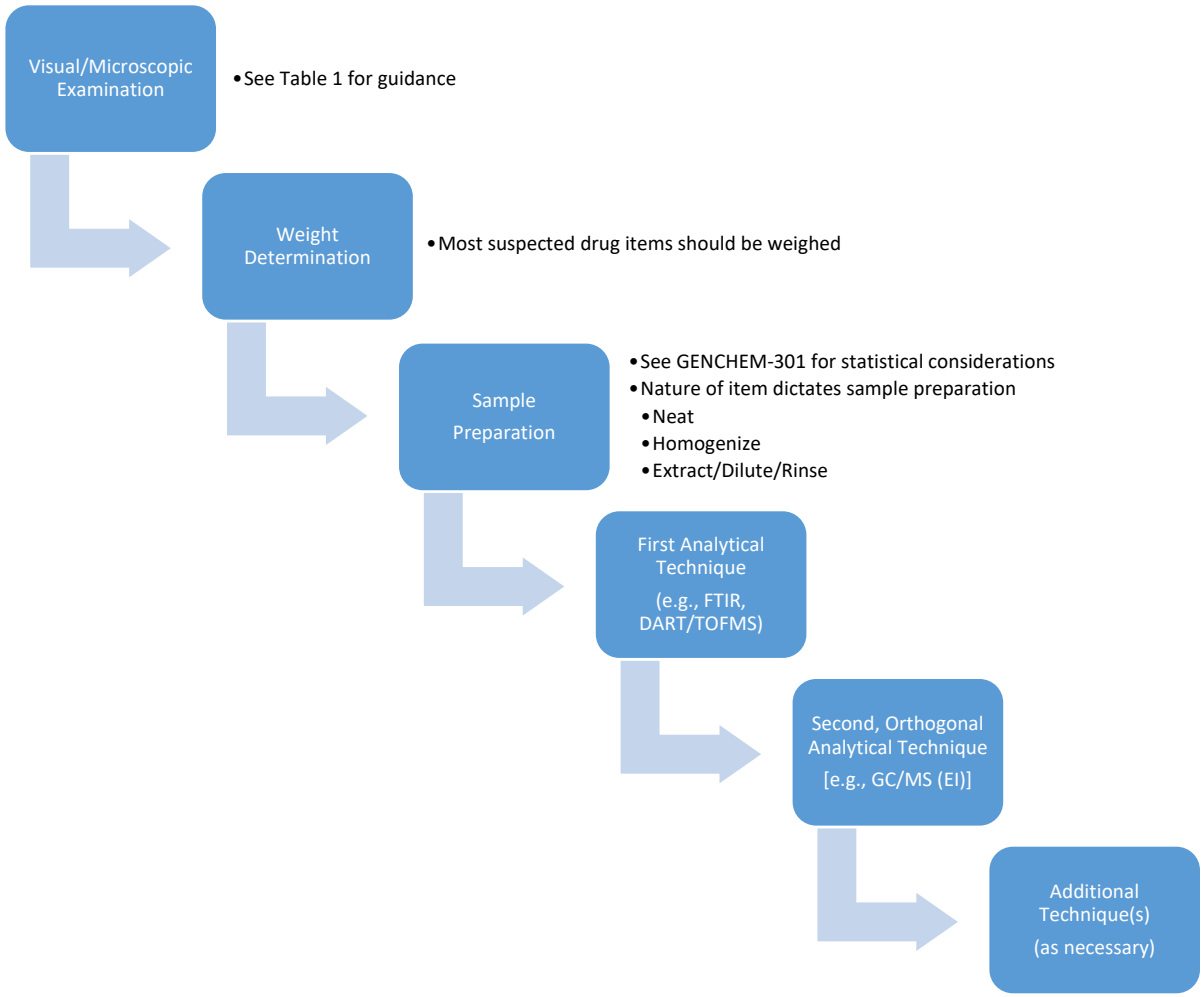
Implementation of the Agriculture Improvement Act of 2018 (DEA 'Interim final rule'). Department of Justice, Drug Enforcement Administration. 21 CFR Parts 1308 and 1312, [Docket No. DEA-500], RIN 1117-AB53

14 REVISION HISTORY

Revision	Issued	Changes
03	4/1/2021	Section 1- added “and authorized” and edited to include only General Chemistry evidence. Section 2- removed ammonium hydroxide, added sodium sulfate (anhydrous). Section 3.2- minor grammatical edit (“into” to “within”). Section 5- added “on a heterogeneous item”. Section 6- added first sentence; step (a)- edited for clarity; step (c)- allowed for test tubes and other containers, as well as changes to reagent and sample amounts; step (f)- added last sentence (and added Appendix A); steps (j) and (k)- added “(or edit the current sequence)”; step (k)- 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 parenthetical details to CU references, added GenChem 34.
04	7/15/2022	Revised to match new format requirements. Integrated content from GENCHEM-502 (Analysis of Tablets and Capsules), GENCHEM-503 (Analysis of Delta-9-tetrahydrocannabinol), and GENCHEM-505 (Synthetic Cannabinoid Analysis); discontinued those technical procedures.

APPENDIX A

General Drug Analysis Flow Chart



APPENDIX B

Acid/Neutral and Alkaline Drug Extractions

Acid/Neutral Drug Extraction

Mix several milligrams of a sample (homogenized if necessary) 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 °C.

Alkaline Drug Extraction

Mix several milligrams of a sample (homogenized if necessary) 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 °C.