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

1 Scope

This procedure allows for the analysis of items suspected of containing the following phosphodiesterase type 5 (PDE-5) inhibitor drugs: sildenafil, tadalafil, vardenafil, udenafil, avanafil, and hydroxyhomo sildenafil (see Appendix A for further details). Additional compounds may be added to this procedure after appropriate validation has been completed. Typical items for analysis include tablets, capsules, liquids, and herbal blends.

This procedure applies to General Chemistry personnel in the Chemistry Unit that are qualified and authorized to examine evidence for the presence of PDE-5 inhibitor drugs.

2 Equipment/Materials/Reagents

- Common laboratory glassware and equipment
- Analytical balance
- Stereo microscope
- Digital microscope
- Fourier Transform Infrared (FTIR) spectrophotometer with Attenuated Total Reflectance (ATR), transmission, or microscope attachments
- Time-of-flight mass spectrometer with direct analysis in real time ionization source (DART/TOFMS)
- Liquid chromatography system with C18 column (or equivalent) coupled to a mass spectrometer (LC/MS) with electrospray ionization (ESI) (e.g., Thermo LTQ, Thermo LTQ OrbiTrap XL, Thermo Exactive OrbiTrap)
- Acetonitrile
- Ammonium hydroxide
- Avanafil
- Deionized water
- Hydroxyhomo sildenafil
- Methanol
- Polyethylene glycol (PEG, 550 average molecular weight)
- Sildenafil citrate
- Tadalafil
- Udenafil
- Vardenafil hydrochloride

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 acetonitrile from the same source and lot and within a similar container used to extract the questioned item(s). It is left to the discretion of the examiner as to what constitutes an adequate Negative Control.

3.2 Positive Controls

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

3.2.1 PDE-5 Positive Control (100 ug/mL)

A PDE-5 inhibitor stock solution is prepared at 1 mg/mL by dissolving 10 mg of the appropriate PDE-5 inhibitor reference material in 10 mL of acetonitrile. The stock solution is then diluted 1:10 with acetonitrile to prepare the 100 ug/mL PDE-5 positive control solution. Store the solutions in glass containers in a freezer.

3.2.2 PDE-5 Positive Control (10 ug/mL)

A 10 ug/mL PDE-5 inhibitor positive control solution for LC/MS is prepared by 1:10 dilution of the 100 ug/mL PDE-5 inhibitor positive control solution with acetonitrile. Store the solutions in glass containers in a freezer.

4 Sampling

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

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

5 Procedure

5.1 Tablets and Capsules

a. Visually examine each item and record relevant information such as number of tablets/capsules, size, shape, color, score marks, and/or logo/imprint information.

b. If the total weight is requested, or is otherwise relevant, use a traceable analytical balance to record the total weight of each type of tablets/capsules.

c. Search 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 may be used in combination with the tablet/capsule weight to determine the amount of material necessary to yield the desired amount of active ingredient for extraction and solution preparation. Note- counterfeit items may contain different ingredients and/or dosages.

d. If statistical sampling is required, refer to *Sampling Guidelines for Bulk Materials and Multi-Unit Populations* (GenChem 21).

e. Homogenize a tablet using a mortar and pestle. If possible, homogenize one half of the tablet while retaining the other half intact. Weigh and transfer a portion of the homogenized tablet to a labeled test tube.

f. Open a capsule to remove a portion of the contents and homogenize with a mortar and pestle if heterogeneous. Weigh and transfer a portion of the contents to a labeled test tube.

g. Use an empty, labeled test tube as a Negative Control.

h. Proceed to section 5.3.

5.2 Powders, Liquids, and Other Items

a. Visually examine each item and record relevant information.

b. Use a traceable analytical balance to record the total weight of a solid item.

c. Use the item's container to estimate the total volume of a liquid item. If the item's container does not have volume information on it, or if a more accurate volume is

necessary, transfer the liquid to a clean graduated cylinder, beaker, or other container using appropriate safety precautions. Measure and record the volume.

d. Homogenize a portion of a solid item with a mortar and pestle. Weigh and transfer a portion of the homogenized solid to a labeled test tube.

e. Vortex mix a liquid sample and use a pipette to transfer a known volume to a labeled test tube. Record the volume transferred.

f. Use an empty, labeled test tube as a Negative Control.

g. Proceed to section 5.3.

5.3 Instrumental Examination Techniques

a. Analyze each sample directly by FTIR. Samples are typically analyzed on the ATR accessory, but may also be analyzed in transmission mode on the bench or microscope. Spectral subtraction may be necessary to observe PDE-5 inhibitor drugs. Preparations that consist of less than ~ 1-5% of active ingredient typically do not provide a strong enough signal to be observed.

b. Prepare each sample in acetonitrile to achieve a PDE-5 inhibitor concentration of ~ 100 ug/mL. If the dosage information is unknown for a sample and the FTIR spectrum cannot be used to estimate the PDE-5 inhibitor concentration, prepare a solid sample at ~ 10 mg/mL or a liquid sample at a dilution of ~ 1:10 in acetonitrile. Add the same volume of solvent to the Negative Control(s) test tube(s).

c. Analyze the Negative Control(s), questioned item(s), and the applicable 100 ug/mL PDE-5 Positive Control(s) by DART/TOFMS in positive ionization mode. The closed end of a glass capillary is typically used to introduce the sample to the DART source. As an alternative to step (b), a neat powder may be sampled with a glass capillary wetted with deionized water; or a neat liquid may be sampled with a glass capillary. In these instances a blank glass capillary (wetted with deionized water; or dry, respectively) will be analyzed in the same collection file as a Negative Control. PEG will be analyzed with each data collection file to allow for mass-to-charge correction. Items that are negative by DART/TOFMS do not need to be analyzed by LC/MS (ESI) unless lower concentrations of PDE-5 inhibitors need to be ruled out.

d. Prepare each sample in acetonitrile to achieve a PDE-5 inhibitor concentration of ~ 1 to 10 ug/mL using the dosage information (or the DART/TOFMS results for items without

dosage information). Filter the Negative Control(s) and questioned item(s) with 0.2 um PTFE syringe filters that have been pre-rinsed with acetonitrile. Collect the filtrates in new, labeled test tubes.

e. Analyze the filtered Negative Control(s), filtered questioned item(s), and the applicable 10 ug/mL PDE-5 Positive Control(s) by LC/MS (ESI). Incorporate acetonitrile blanks between each sample.

6 Calculations

Following is an example calculation for preparing a 100 ug/mL extraction solution of sildenafil from a tablet with an indicated dosage of 100 mg.

$$\frac{100\ mg\ sildenafil\ citrate}{611.7\ mg\ tablet\ weight} \times \frac{474.58\ mg\ sildenafil}{666.70\ mg\ sildenafil\ citrate} \times 100 = 11.6\ wt.\%\ sildenafil$$

$$\frac{X\ mg\ tablet\ \times 11.6\ wt.\%\ sildenafil}{10\ mL\ acetonitrile} = \frac{0.1\ mg\ sildenafil}{1\ mL\ acetonitrile}$$

$$X\ mg\ tablet = \frac{0.1\ mg\ sildenafil}{1\ mL\ acetonitrile} \times \frac{10\ mL\ acetonitrile}{11.6\ wt.\%\ sildenafil}$$

$$X\ mg\ tablet = 8.6\ mg$$

For this example, weigh and transfer 8.6 mg of the homogenized item to a labeled test tube, then add 10 mL acetonitrile to yield a sildenafil solution of ~ 100 ug/mL (assuming 100% recovery).

7 Measurement Uncertainty

Not applicable

8 Instrumental Conditions

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

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

8.1 Liquid Chromatography/Mass Spectrometry (LC/MS)

8.1.1 Liquid Chromatography Parameters

Mobile Phase Compositions		**Flow Parameters**			**Column Parameters**	
A: 0.03% ammonium hydroxide, 5% methanol (v/v) in deionized water		total flow = 0.30 mL/min			type	C18
		time (min)	% A	% B	length	150 mm
B: 0.03% ammonium hydroxide (v/v) in methanol		0	50	50	internal diameter	2.1 mm
		2.0	50	50	particle size	5 um
		7.0	5	95	temperature	30 ºC
Autosampler		12.0	5	95		
temperature	15 ºC	13.0	50	50		
injection volume	5 uL	18.0	50	50		
		total run time = 18 min.				

8.1.2 Mass Spectrometer Parameters

8.1.2.1 Full Scan MS Only

Duration = 11.00 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.	
Scan Event #1	
Ionization mode	ESI (+)
Scan mode	Full scan MS
Scan range	130-550 *m/z*

8.1.2.2 Data Dependent MS

Duration = 11.00 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.	
Scan Event #1	
Ionization mode	ESI (+)
Scan mode	Full scan MS
Scan range	130-550 *m/z*
Scan Event #2	
Ionization mode	ESI (+)
Scan mode	MS/MS, most intense ion from Scan Event #1
Parent mass list	389.90, 475.11, 484.06, 489.13, 505.14, 517.15
Min. signal required	5000.0
Collision energy	30 %
Product scan range	80-1095 *m/z*
Q	0.250
Time	30.000
IsoW	2.0

9 Limitations

The following conclusions apply to the analysis of PDE-5 inhibitor 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

Blok-Tip, L., Zomer, B., Bakker, F., et. al., "Structure elucidation of sildenafil analogues in herbal products", *Food Additives & Contaminants*, Volume 21, Issue 8, pp 737-748.

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.

Zhu, X., Xiao, S., Chen, B., et. al., "Simultaneous determination of sildenafil, vardenafil and tadalafil as forbidden components in natural dietary supplements for male sexual potency by high-performance liquid chromatography-electrospray ionization mass spectrometry", *Journal of Chromatography A*, 1066 (2005), pp 89-95.

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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.

Balayssac, S., Trefi, S., Gilard, V., et. al., "2D and 3D DOSY 1H NMR, a useful tool for analysis of complex mixtures: Application to herbal drugs or dietary supplements for erectile dysfunction", *Journal of Pharmaceutical and Biomedical Analysis 50*, (2009), pp 602-612.

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

General Chemistry Instrument Parameters; FBI Laboratory Chemistry Unit – General Chemistry SOP (GenChem 34)
Chemistry Unit (CU) FBI Approved Standards for Scientific Testimony and Report Language for General Chemistry – General Chemistry SOP (GenChem 32)

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

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

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

FBI Laboratory Safety Manual

Rev. #	Issue Date	History
1	06/02/20	Removed previous sections 1 (Introduction), 3 (Principle), 4 (Specimens), 7 (Calibration), and 11 (Decision Criteria, including Tables 1 and 2); renumbered sections accordingly. LC/MS parameters changed for efficiency and better separation of avanafil, hydroxyhomo sildenafil, and avanafil. 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 section 3 to add detail; changed formatting. Added content to section 4 (Sampling) and updated reference. Edited content of section 5 for clarity and added content in section 5.2. Added example calculation to section 6. Changed new section 7 title from 'Uncertainty of Measurement'. Section 8 edited to refer to GenChem 34 for instruments except LC/MS. Section 9 edited to include conclusion statements and references to ASSTR, ULTR, etc. Updated references in section 11.
2	04/01/21	Section 1- added "and authorized". Sections 5.1, 5.2, and 5.3- added line spacing between each step for ease of reading (did not mark this with change indicators as content did not change). Section 9- added last sentence.

Approval

Redacted - Signatures on File

Chemistry Unit Chief: Date: 03/31/2021

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