Designation: E3290 – 21

Standard Test Method for Establishing Performance of Equipment and Assays for Field Detection of Fentanyl and Fentanyl-Related Compounds[1]

This standard is issued under the fixed designation E3290; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

INTRODUCTION

As a result of the widespread use of synthetic opioids, first responders and other response personnel may encounter fentanyl and fentanyl-related compounds while working in the field. To ensure that first responders can identify these compounds in the field, it is critical for end-users to understand the performance metrics of detection equipment and assays to ensure these products meet the intended end-user needs. It is also necessary to understand the potential effects on detection systems when common cutting agents and other interferents, including drugs (for example, heroin), are present in samples.

The test methods presented in this standard are intended for equipment and assays commonly employed in the field for the detection of suspected drugs that may contain fentanyl and fentanyl-related compounds. The test methods presented here are not intended for laboratory equipment, but rather for testing of field detection equipment in a laboratory setting. The test methods are also not intended to reflect testing approaches that would be performed in the field (for guidance on using field detection equipment in the field, the user is referred to the companion ASTM standard Guide E3289). This test method is a companion standard to Specification E3243. The specification describes a statistical testing approach to quantify performance and defines test sample compositions and amounts. However, Specification E3243 does not describe in detail sample preparation methods or specific protocols for conducting testing with different types of instruments and assays. These methods and protocols are described in detail in this test method.

1. Scope

1.1 *General:*

1.1.1 This test method provides a procedure for characterizing the performance of field portable fentanyl detection equipment and assays when utilizing the test samples and statistical considerations described in Specification E3243.

1.1.2 This test method describes sample preparation and analysis protocols to use when characterizing the performance of various types of field fentanyl detection equipment or assays in a laboratory environment including gas chromatography/mass spectrometry (GC/MS), high pressure mass spectrometry (HPMS), ion mobility spectrometry (IMS), Fourier Transform Infrared (FTIR) spectroscopy, Raman spectroscopy, colorimetric assays, and immunoassays.

1.1.3 The intent of this test method is to analyze samples in a manner that is analogous to how they are analyzed in the field by Federal and State/Local/Tribal/Territorial (SLTT) law enforcement and first responders, but under much more controlled and reproducible conditions than those that would generally be achievable when conducting field testing.

1.2 *Units:*

1.2.1 When creating test sample mixtures, all concentrations are stated as weight/weight percent (mg/mg) for solid sample mixture test samples, and weight/volume (mg/mL) for solid and liquid test samples that are dissolved in a solvent. When creating diluted liquid test samples (for example, for detection of compounds solubilized in solvent prior to analysis), all concentrations are stated as volume/volume percent (for example, µL/mL).

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

[1] This test method is under the jurisdiction of ASTM Committee E54 on Homeland Security Applications and is the direct responsibility of Subcommittee E54.01 on CBRNE Detection and Decontamination.

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1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E2771 Terminology for Homeland Security Applications

E3131 Specification for Nucleic Acid-Based Systems for Bacterial Pathogen Screening of Suspicious Visible Powders

E3243 Specification for Field Detection Equipment and Assays Used for Fentanyl and Fentanyl-Related Compounds

E3289 Guide for Using Equipment and Assays for Field Detection of Fentanyl and Fentanyl-Related Compounds

2.2 *Eurachem Standard:*[3]

Eurachem/CITAC Guide CG 4 Quantifying Uncertainty in Analytical Measurement, Third edition (2012)

2.3 *ISO Standard:*[4]

ISO 17034 General requirements for the competence of reference material producers

2.4 *OSHA Standards:*[5]

29 CFR 1910.132 OSHA Personal Protective Equipment Standard

29 CFR 1910.134 OSHA Respiratory Protection Standard

3. Terminology

3.1 *Definitions:*

3.1.1 *accuracy, n*—closeness of agreement between a test result and the accepted reference value. **E2771**

3.1.2 *assay, n*—quantitative or qualitative test used to determine the presence or absence of a chemical compound.

3.1.3 *bulk sample, n*—total sample amount (including the sum of target and non-target compounds) that is visible to the naked eye.

3.1.3.1 *Discussion*—The amount of sample available for testing bulk samples according to this standard is >1 µg, but ≤10 mg.

3.1.4 *confidence interval, CI, n*—range of values created using a procedure that, when repeated many times, on distinct data sets, generated from the same underlying stochastic process, will bracket the true measure of performance, such as probability of detection (POD), the proportion of times stated. **E3131**

3.1.5 *confidence level, CL, n*—probability value associated with a CI; the percentage of intervals that can be expected to include the true population parameter over time.

3.1.6 *false negative, n*—failure to detect a compound within a sample when it is present.

3.1.7 *false positive, n*—detection of a compound within a sample when it is not present. **E3131**

3.1.8 *lower confidence bound, LCB, n*—lowest value of a one-sided CI created using a procedure that, when repeated many times on distinct data sets generated from the same underlying stochastic process, will include the true measure of performance a proportion of times equal to the stated probability. **E3131**

3.1.8.1 *Discussion*—The LCB ensures that the POD attains a satisfactory value for the CL selected and determines the minimum number of samples that shall be analyzed.

3.1.9 *measurement process, n*—step, or series of systematic steps, used to detect a material or determine if a system or instrument performs as intended. **E3131**

3.1.10 *non-target compound, n*—collection of diluents/cutting agents, dyes/colorants, and other drugs that are not the target compound, which in this case is fentanyl or any fentanyl-related substance.

3.1.10.1 *Discussion*—Non-target compounds should not give a positive test result for fentanyl or fentanyl-related substances; this is considered a false positive result. Ideally, all non-target compounds should result in negative detection results for fentanyl and fentanyl-related compounds.

3.1.11 *operator, n*—person operating an on-site chemical assessment technology.

3.1.11.1 *Discussion*—This definition differs slightly from that in Specification E3131 as it applies to the chemical detection technology referred to in this test method.

3.1.12 *precursor, n*—chemical compound that is used in the synthesis of fentanyl or fentanyl-related compounds.

3.1.13 *probability of detection, POD, n*—proportion of positive analytical outcomes for a qualitative method for a given matrix at a given concentration.

3.1.14 *reach-back support, n*—service that allows equipment users in the field to be in real-time contact with subject matter experts (SMEs) to provide advice an assessment of analysis results, including, but not limited to identification of one or more substances in an unknown sample.

3.1.14.1 *Discussion*—This service is often available 24 h a day/7 days per week via paid subscription through an equipment (instrument or assay) manufacturer. For example, a field chemical detection instrument like a Raman spectrometer may be used to scan an unknown sample suspected of containing narcotics. The user can send the resulting data to reach-back support for review or interpretation. The SMEs providing the support can identify poor quality data and recommend settings for a successful rescan, perform advanced data analysis techniques to identify chemicals present in the sample, and quickly provide decision support capabilities and guidance in response to end-user requests and inquiries. Reach-back services are commonly used for aiding in the interpretation of ambiguous

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from the Eurachem organization, https://www.eurachem.org/index.php/publications/guides/quam.

[4] Available from International Organization for Standardization (ISO), ISO Central Secretariat, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, https://www.iso.org.

[5] Available from Occupational Safety and Health Administration (OSHA), 200 Constitution Ave., NW, Washington, DC 20210, http://www.osha.gov.

data and for confirmation of both positive and negative results (especially those with which decisions of consequence may be taken).

3.1.15 *reference material, n*—substance sufficiently homogenous and stable with respect to one or more specified properties that has been established to be fit for its intended use in the measurement process; properties can be quantitative or qualitative. **ISO 17034**

3.1.16 *saturation, n*—condition in which the detector response no longer increases with increased sample concentration.

3.1.16.1 *Discussion*—This can occur when there is too much sample introduced to an instrument or assay. For optical detection instruments, too much exposure to light, including ambient light, can saturate the detector.

3.1.17 *sensitivity, n*—change in the response of a measuring instrument divided by the corresponding change in the stimulus. **Eurachem/CITAC Guide CG 4:2012**

3.1.18 *specificity/selectivity, n*—ability of a measurement procedure to determine accurately and specifically the analyte of interest in the presence of other components in the sample matrix under the stated conditions of the test.
Eurachem/CITAC Guide CG 4:2012

3.1.19 *target compound, n*—any of a collection of fentanyl and fentanyl-related compounds.

3.1.20 *test module, TM, n*—set of samples with particular characteristics used to establish the detection technology performance for a given type of sample.

3.1.20.1 *Discussion*—TMs include target compound samples at three different percent sample compositions (≥95 %, 10 %, and 1 %), non-target samples that do not contain fentanyl or fentanyl-related compounds, and fentanyl synthesis precursors or other compounds used for synthesis. TMs are given in Annex A1 of Specification E3243.

3.1.21 *test sample, n*—amount and identity of a particular substance (target and non-target compounds) prepared for testing.

3.1.22 *trace sample, n*—total sample amount, including target and non-target compounds, ≤1 µg used for testing.

3.1.22.1 *Discussion*—In this test method, a trace sample is intended to represent a sample not visible to the naked eye, a situation commonly encountered by first responders. For practical purposes, it is assumed that the sizes represented by bulk and trace samples sufficiently cover the range of situations encountered in the field where the testing equipment may be used.

3.2 *Acronyms:*

3.2.1 *ATR*—attenuated total reflectance.

3.2.2 *CDC*—Centers for Disease Control.

3.2.3 *CFR*—Code of Federal Regulations.

3.2.4 *CI*—confidence interval

3.2.5 *CL*—confidence level.

3.2.6 *FEMA*—Federal Emergency Management Agency.

3.2.7 *FTIR*—Fourier transform infrared (also known as Fourier transform infrared spectroscopy).

3.2.8 *GC*—gas chromatography.

3.2.9 *GC/MS*—gas chromatography/mass spectrometry.

3.2.10 *HPMS*—high pressure mass spectrometry.

3.2.11 *IMS*—ion mobility spectrometry.

3.2.12 *ISO*—International Organization for Standardization.

3.2.13 *LCB*—lower confidence bound.

3.2.14 *MS*—mass spectrometry.

3.2.15 *NIOSH*—National Institute for Occupational Safety and Health.

3.2.16 *OSHA*—Occupational Safety and Health Administration.

3.2.17 *POD*—probability of detection.

3.2.18 *PPE*—personal protective equipment.

3.2.19 *RSD*—relative standard deviation.

3.2.20 *SERS*—surface enhanced Raman spectroscopy.

3.2.21 *SLTT*—state, local, tribal, and territorial.

3.2.22 *SME*—subject matter expert

3.2.23 *TM*—test module

3.2.24 *US&R*—urban search and rescue (which may be FEMA or state teams).

4. Summary of Test Method

4.1 Test samples containing known concentrations/amounts of target and non-target analyte as defined in Annex A1 of Specification E3243 are selected, mixed, and prepared for testing.

4.2 Instrument or assay start-up, preparation, system checks, and calibrations are performed per manufacturer's instructions and guidance.

4.3 Test samples and control samples are analyzed according to manufacturer's instructions and guidance.

4.4 Required documentation is collected.

4.5 Results are evaluated and recorded.

4.6 Results are reported.

4.7 In this test method, each detection technology is presented in a separate section (Sections 12 – 19). Sections that pertain to all detection technologies include Sections 5 – 11 and Sections 20 and 21.

5. Apparatus

5.1 Appropriate personal protective equipment (PPE), including appropriate nitrile gloves.[6]

5.2 Analytical balance capable of weighing to 0.0001 g.

5.3 Chemical fume hood suitable for working with organic solvents and highly toxic material such as fentanyl in liquid and solid form.

[6] See https://www.tandfonline.com/doi/full/10.1080/15459624.2020.1784426.

5.4 Mixing apparatus and containers.[7]

5.5 Calibrated dispensing devices to accurately and consistently deliver necessary aliquots between 10.00 mL and 1.00 µL.

5.6 Instrument or assay kit in operational readiness.

5.7 *For Raman Instruments:*

5.7.1 Shroud (for example, black or light blocking cloth, if supplied or required by manufacturer).

5.8 *Reagents and Materials:*

5.8.1 See Annex A1 of Specification E3243 for a listing of target and non-target chemical compounds needed for testing.

5.8.2 Ultrapure water.

5.8.3 Ethanol.

5.8.4 Methanol.

5.8.5 Other solvents as indicated by an instrument or assay manufacturer.

5.8.6 Volumetric flasks.

5.8.7 Swabs.

5.8.8 Alcohol wipes.

6. Hazards

6.1 Take all necessary precautions when working with fentanyl and fentanyl-related compounds, semi-synthetic opioids, other drugs, and other hazardous chemicals contained in the target, non-target, and precursor test panels. Implement best practices for working with chemicals, including use of proper PPE.[7] Fentanyl and fentanyl-related compounds can be extremely hazardous if appropriate handling and PPE are not adhered to. In general, an exposure of 2 to 3 mg of fentanyl or 20 µg of carfentanil can be lethal if inhaled, ingested, or absorbed through mucous membranes. Dermal exposure is not typically associated with significant hazards. Seven grains of table salt represent about 1 mg.

6.2 Safety Data Sheets for all chemicals (targets, non-targets, precursors, solvents) should be consulted before use. The user of this test method should also be aware of the hazards associated with the operation of the chosen instruments or assays.

6.3 Some instruments may contain hazards related to radioactive materials or lasers and some assays contain hazardous chemicals. Consult the manufacturer-provided user manual and training materials for hazards specific to the instrument or assay being used.

[7] See https://www.cdc.gov/niosh/topics/fentanyl/risk.html.

7. Required Documentation

7.1 Include, at a minimum, enough information in the documentation of all chemical compounds used in testing and sample preparation and dilution (for example, solvents) to trace the material used to its source, including chemical manufacturer, catalog or product number or both, lot number, purchase date, and any manufacturer-provided quality information/certificates.

7.2 Record all calculations (for example, for weighing and diluting).

7.3 Prepare any instrument or assay for testing in accordance with manufacturer's guidance, including any calibration, tuning, or operational checks. Record all protocols used and results obtained.

7.4 Record control sample identity and control sample results and all TM samples associated with those controls (see 8.9).

7.5 Save all associated raw data and data analysis/reporting outputs provided to the user after testing a TM sample or control sample.

8. Selecting and Preparing Test Samples

8.1 Select applicable TMs and prepare samples containing known concentrations and amounts of target and non-target compounds. See Table 1, Table 2, and Table 3, and Annex A1 in Specification E3243.

8.2 Prepare bulk samples (>1 µg but <10 mg) in suitably sized sealable containers. For individual samples in each TM, 2 mL glass vials are recommended. For Raman measurements, flat bottom "33 expansion" glass vials are recommended; curved and thick bottom glass vials should be avoided.

8.3 Prepare trace samples starting with bulk sample amounts as suggested in Table 1 at the time of testing following manufacturer's guidance specific to the instrument/assay being evaluated for further sample preparation/dilution (for example, solvent dissolution followed by serial dilution).

8.3.1 Smaller sample amounts may be used subject to guidance from the instrument or assay manufacturer, SMEs, or prior testing knowledge.

8.4 Each sample container in a specific TM should contain the same mass of sample within a 10 % uncertainty (for example, 2 mg ± 0.2 mg); the amounts do not need to be the same for all TMs, as long as the amounts are uniform within a specific TM.

TABLE 1 Recommended Bulk Sample Amounts for Different Detection Technologies

Detection Technology	Recommended Bulk Amount of Sample per Vial
Colorimetric Assay	1 to 2 mg
FTIR Spectroscopy	1 to 3 mg
Gas Chromatography/Mass Spectrometry (GC/MS)	1 to 2 mg
High-Pressure Mass Spectrometry (HPMS)	1 to 2 mg
Immunoassay	1 to 2 mg
Ion Mobility Spectrometry	1 to 2 mg
Raman Spectroscopy	9 to <10 mg
Surface Enhanced Raman Spectroscopy (SERS)	1 to 2 mg

8.5 When preparing TM samples record all sample preparation calculations, mixing calculations, and test data used to qualify accurately prepared mixtures.

8.6 Weigh materials with a balance that can measure ≤2 mg with a maximum 10 % uncertainty.

8.7 *Preparation of Multicomponent TM Samples (TM2, TM3, and TM4):*

8.7.1 Determine how much total mass of each chemical is necessary for the desired mixtures. Calculate the exact mass of each chemical needed to achieve the specified percent composition for a given mixture and prepare enough mixture so that the minimum amount of any one component in the sample mixture is not less than 1 to 2 mg.

8.7.2 A minimal degree of sample processing is necessary to provide results that can be compared among different testing events. Prior to weighing, all solids should be processed through a mesh-60 sieve. Retain all particles smaller than mesh-60 for testing. If necessary, use a mortar and pestle with minimal grinding to reduce the particle sizes sufficiently to pass through the mesh-60 sieve. Do not overgrind as this will result in sample physical properties that do not reflect real-world illicit drug samples.

8.7.3 Weigh out each chemical to within 10 % of the calculated (desired) mass and combine in an appropriate mixing container. Ensure that the balance has a measurement uncertainty of 10 % or less for the smallest mass to be weighed. The smallest or minimum weight for a balance is determined by multiplying the balance's repeatability by two and dividing the result by the desired measurement uncertainty, expressed in decimal form. For example, the minimum weight to obtain at least 10 % uncertainty for a balance with a reported repeatability of 0.04 mg is (0.04 mg × 2)/0.1 or 0.8 mg.

8.7.4 Mix the combined chemicals using an appropriate mixer and mixing settings.[8] Handle the final mixed sample with utmost care to avoid unnecessary shaking or jarring that may result in the separation of mixture components.

8.7.5 Verify the sample mixture is sufficiently homogeneous by analyzing five subsamples using a quantitative analytical technique such as solvent dissolution followed by liquid chromatography with ultraviolet or mass spectrometric detection (LC/UV or LC/MS). Other techniques are acceptable. Collect five samples of at least 2 mg each from different locations in the final mixed sample. This can be accomplished by removing the mixture from the mixing container and placing it onto a clean surface (for example, a metal plate, silicon wafer, or weighing paper). Dissolve each of the five aliquots in an appropriate volume of solvent (5 % or less volumetric uncertainty) and analyze using one or more analytical methods that can determine absolute or relative concentrations (or signal intensities) for all mixed components.

8.7.5.1 The quantitative values obtained from the analysis should be compared to values obtained from standard solutions.

8.7.5.2 Standard solutions are prepared by dissolving each individual chemical component of the mixture sample in an appropriate volume of solvent (5 % or less volumetric uncertainty) to produce a "parent" solution. Parent solutions are diluted prior to quantitative analysis such that the concentrations or signal intensities of the compounds are similar (within a factor of two) to those expected for the five aliquots of the mixture.

8.7.5.3 Calculate the relative standard deviation (RSD) for reported values of each component among the five samples. The RSD is calculated for each component by dividing the standard deviation of the relative concentration or relative intensity of the five samples by its mean and multiplying the result by 100 %. The error in the mean concentration for each component is determined using the standard solution's relative concentration or relative signal intensity. Both error and RSD values of 20 % or less indicate that adequate mixing was achieved.

8.7.5.4 If the mixture is acceptable for use in a TM, weigh out material into 2 mL vials following the guidelines of Table 1 for the instrument or assay to be tested.

8.8 *Analysis and Reporting of Control Samples:*

8.8.1 Throughout the course of testing, analyze available positive and negative control samples using a testing procedure recommended by the manufacturer to demonstrate that the instrument or assay is operating correctly, and that the testing environment is free from contamination. Test the specific control samples recommended by the manufacturer (note that in some cases the manufacturer's instructions may only call for one type of control sample – either positive or negative; follow manufacturer's guidelines).

8.8.2 Test positive and negative control samples at least once per 10 test samples analyzed or at least once each day that tests are performed, whichever is greater. Follow manufacturer's guidance.

8.8.3 Do not include results from control samples in any calculation of LCB or POD.

8.8.4 Stop testing if failures with controls, including, but not limited to, indication of contamination occur. Testing shall remain on hold until a cause for the failure, or failures, has been found and corrected. Invalidate all TM sample testing results associated with the failed controls. Repeat the invalidated tests, but only after the cause of the failure has been identified and corrected, and the instrument/assay has been cleared for performance following the appropriate manufacturer's protocols.

8.9 *Analysis and Reporting of Test Samples:*

8.9.1 Depending on the number of samples in each TM chosen for testing (31, 14, or 4), testing may cease after a certain number of failures for that TM as the minimum acceptable performance level of 0.85/80 % LCB/CL would not be attainable (see Section 5 in Specification E3243). However, testing may continue until all the samples in the selected TM have been tested if desired. In this case, testing of all samples planned for allows for obtaining a more precise estimate of performance even though it will be below 0.85/80 % LCB/CL.

8.9.2 For instruments that have reach-back support, two test results are obtained (see Section 21):

[8] LabRam I, Resodyn Acoustic Mixers, Butte, MT, USA. Settings: 75G for 5 min mixing time. Use 2 mL GC vials with 500 µL insert. The make, model and settings are provided as examples that were tested and shown to produce well-mixed multicomponent samples. Other products that can be shown to produce well-mixed multicomponent samples when following the protocol in 8.7 may also be used.

8.9.2.1 The direct readout result, and

8.9.2.2 The result provided by reach-back.

8.9.3 If 4 samples are chosen to be tested in an applicable TM, testing may cease when a direct readout result fails AND when any result provided by reach-back fails;

8.9.4 If 14 samples are chosen to be tested in an applicable TM, testing may cease when a single direct readout result fails AND when a result provided by reach-back fails;

8.9.5 If 31 samples are chosen to be tested in an applicable TM, testing may cease when more than three direct readout results fail AND when more than three results provided by reach-back fail.

9. Preparation of Apparatus

9.1 Prepare instruments or assays according to manufacturer's guidance, including considerations of temperature, ambient light and brightness, humidity, dust, and other environmental factors.

9.2 Ensure analytical balance and pipettes are calibrated.

9.3 Ensure glassware and any weighing accessories (for example, spatula) are clean.

10. Calibration and Standardization

10.1 Ensure each instrument is calibrated and not in need of service per manufacturer's instructions.

10.2 Ensure assays are not expired and packaging or storage has not been compromised.

11. Conditioning

11.1 Consult user manuals for any instrument or test-specific setup features.

12. Gas Chromatography/Mass Spectrometry (GC/MS) Test Procedure

12.1 Following procedures outlined in Section 8, choose to test bulk amounts of test sample (>1 µg but <10 mg, note that samples may need to be diluted to avoid saturating the detector) or trace amounts of test sample (≤1 µg target compound), choose the test module (TM1, TM2, TM3, TM4, and TM5), select the number of samples to be tested (31, 14, or 4), and choose the number of different sample types to test (14 or 4; for clarification, see 4.13 in Specification E3243).

12.2 Refer to user manual and training materials for the specific instrument being tested.

12.3 Power on instrument and allow it to perform boot sequence, including allowing it to reach operating temperature.

12.4 Log in and perform system checks as required.

12.5 Install appropriate vapor or trace sampling accessory for sample to be tested.

12.6 Ensure that target compounds are present in the library to be used for analysis.

12.7 Don appropriate PPE, including powder-free nitrile gloves.

12.8 Analysis of bulk samples can be done in GC/MS mode or using just MS for vapor detection using a sampling wand or handheld probe. Sampling directly from solids may require additional accessories or software methods.

12.8.1 *Bulk Sample Testing in GC/MS Mode:*

12.8.1.1 Dissolve the sample in manufacturer-recommended solvent to a concentration of 1 to 10 mg/mL (1 to 10 µg/µL; weight/volume) using volumetric glassware or pipettes that provide a volumetric uncertainty of 5 % or better, and an analytical balance that has a measurement uncertainty of 10 % or better (never measure less than the minimum manufacturer-recommended weight for a balance). Follow manufacturer's recommendations for further sample handling (for example, shake to mix and particulate settling) and further dilution, if necessary, so as not to introduce a concentration that will saturate the instrument.

12.8.1.2 Once further sample handling and dilution has occurred, inject 1 µL of solution into instrument using a standard 5-µL GC syringe if there is no other specific guidance.

12.8.2 *Bulk Sample Testing in Vapor Detection Mode:*

12.8.2.1 For high vapor pressure liquids and solids, survey mode can be used for quick identification using a sampling wand or handheld probe and passing the probe over an open vial of test sample, taking care not to saturate the instrument.

12.8.2.2 If sampling in vapor mode, slowly approach sample with wand or probe until sufficient signal is observed.

12.8.2.3 Sampling directly from low vapor pressure solids may require additional accessories or software methods. Note that the intent of this test method is to analyze samples in a manner that is analogous to how they are analyzed in the field by Federal and SLTT law enforcement and first responders. Some sampling approaches, while available, may not reflect how law enforcement and first responders actually use this equipment, in which case they should not be used. Consult with SMEs and equipment end-users.

12.8.2.4 Perform scan according to manufacturer's instructions, appropriate for sample type and identification mode.

12.8.3 *Trace Sample Testing in GC/MS Mode:*

12.8.3.1 Dissolve the sample in manufacturer-recommended solvent to a concentration of 0.1 to 1 mg/mL (0.1 to 1 µg/µL; weight/volume) using volumetric glassware or pipettes that provide a volumetric uncertainty of 5 % or better, and an analytical balance that has a measurement uncertainty of 10 % or better (never measure less than the minimum manufacturer-recommended weight for a balance). Shake to mix.

12.8.3.2 1 µL of this solution is injected (no more), resulting in the required introduction of no more than 1 µg of sample per the definition of trace sample. Alternative concentrations and volumes are acceptable (for example, 2 µL injection of a 0.5 µg/µL solution) as long as no more than 1 µg of sample is introduced. Introduction of <1 µg of sample is acceptable. Note that for TM2 (10 % target compound), 1 µg of sample equates to 100 ng of target compound and for TM3 (1 % target compound), 1 µg of sample equates to 10 ng of target compound.

12.8.3.3 Perform scan according to manufacturer's instructions, appropriate for sample type and identification mode.

12.9 *Data Inspection and Analysis:*

12.9.1 For TM1, TM2, and TM3, if the target compound cannot be identified as fentanyl or a fentanyl-related compound, the analysis of the test sample has failed (see 8.9). Retest only if an error has been made by the testing entity or the instrument has malfunctioned based on the failure of control samples (see 8.8).

12.9.2 For TM4, if the result indicates the presence of fentanyl or a fentanyl-related compound, the analysis of the test sample has failed (see 8.9). Retest only if an error has been made by the testing entity or the instrument has malfunctioned based on the failure of control samples (see 8.8).

12.9.3 For TM5, if the identification of the test sample does not indicate the presence of the precursor-related compound, the analysis of the test sample has failed.

12.9.4 If the instrument is working below optimal conditions, maintenance procedures or recalibration may be required. Consult manufacturer if necessary, to troubleshoot. Conduct all testing under homogeneous instrument conditions. Maintenance or recalibration procedures may affect instrument performance and effectively result in different testing conditions for samples tested before and after the procedure occurs, which may invalidate the results.

13. High-Pressure Mass Spectrometry (HPMS) Test Procedure

13.1 Following procedures outlined in Section 8, choose to test bulk amounts of test sample (>1 µg but <10 mg; note that samples may need to be diluted to avoid saturating the detector) or trace amounts of test sample (≤1 µg target compound), choose the test module (TM1, TM2, TM3, TM4, and TM5), select the number of samples to be tested (31, 14, or 4), and choose the number of different sample types to test (14 or 4; for clarification, see 4.13 in Specification E3243).

13.2 Refer to user manual and training materials for specific instrument being tested.

13.3 Power on instrument and allow it to perform boot sequence, including allowing it to reach operating temperature.

13.4 Log in and perform any required system checks as required.

13.5 Install appropriate vapor or dry swipe accessory for sample to be tested.

13.6 Log in and perform system checks as required.

13.7 Ensure that target compounds are present in the library to be used for analysis.

13.8 Don appropriate PPE, including powder-free nitrile gloves.

13.9 Analysis of bulk samples can be done in thermal desorption mode (requires solubilization of sample and application to a swipe) or in the vapor detection "sniffer" mode.

13.9.1 Bulk sample testing in thermal desorption mode:

13.9.1.1 Dissolve the sample in manufacturer-recommended solvent to a concentration of 1 to 10 mg/mL (1 to 10 µg/µL; weight/volume) using volumetric glassware or pipettes that provide a volumetric uncertainty of 5 % or better, and an analytical balance that has a measurement uncertainty of 10 % or better (never measure less than the minimum manufacturer-recommended weight for a balance). Follow manufacturer's recommendations for further sample handling and dilution, if necessary, so as not to introduce a concentration that will saturate the instrument.

13.9.1.2 Spike a manufacturer-recommended volume (typically 1 to 2 µL) of the solubilized dilute sample onto an appropriate dry swipe using a standard 5-µL GC syringe.

13.9.1.3 Perform scan according to manufacturer's instructions, appropriate for sample type and identification mode.

13.9.2 Bulk sample testing using vapor detection mode:

13.9.2.1 For high vapor pressure liquids and solids, vapor detection mode can be used by passing the instrument's vapor inlet over an open vial of test sample, taking care not to saturate the instrument.

13.9.2.2 If sampling in vapor mode, slowly approach sample with instrument until sufficient signal is observed.

13.9.2.3 Perform scan according to manufacturer's instructions, appropriate for sample type and identification mode.

13.9.3 Trace sample testing in thermal desorption mode:

13.9.3.1 Dissolve the sample in manufacturer-recommended solvent to a concentration of 1 mg/mL (1 µg/µL) using volumetric glassware or pipettes that provide a volumetric uncertainty of 5 % or better, and an analytical balance that has a measurement uncertainty of 10 % or better (never measure less than the minimum manufacturer-recommended weight for a balance).

13.9.3.2 Dilute the 1 µg/µL solution by a factor of 4 (5 % uncertainty or better) to prepare a 0.25 µg/µL dilution. Spike 4 µL of the diluted sample onto an appropriate dry swipe using a standard 5-µL GC syringe. Spiking smaller volumes onto a swipe or use of different dilutions is acceptable as long as no more than 1 µg of total sample amount is introduced. Note that for TM2 (10 % target compound), 1 µg of sample equates to 100 ng of target compound and for TM3 (1 % target compound), 1 µg of sample equates to 10 ng of target compound.

13.9.3.3 Allow the swipe to dry for at least 30 s, or until dry, and then analyze.

13.9.3.4 Final test samples can be generated through multiple rounds of swab to swab serial dilution to reach appropriate sample concentration if necessary.

13.9.3.5 Perform scan according to manufacturer's instructions, appropriate for sample type and identification mode.

13.9.4 Review scan results and interpret according to manufacturer's guidance.

13.10 *Data Inspection and Analysis:*

13.10.1 For TM1, TM2, and TM3, if the target compound cannot be identified as fentanyl or a fentanyl-related compound, the analysis of the test sample has failed (see 8.9). Retest only if an error has been made by the testing entity or the instrument has malfunctioned based on the failure of control samples (see 8.8).

13.10.2 For TM4, if the result indicates the presence of fentanyl or a fentanyl-related compound, the analysis of the