Instrument Parameters and Reagent Preparation

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1 INTRODUCTION

Explosives chemistry utilizes analytical instruments to analyze evidence containing explosives, residues, precursors, and general unknown materials. This procedure describes basic operation of the instruments as well as specific parameters and method information for the analysis of different types of samples.

A performance verification standard or testmix is used during the Quality Assurance and Control (QA/QC) performance monitoring process. It will be analyzed and evaluated prior to the analysis of evidence. Testmixes may contain a substance that is only used to verify instrument performance and is not expected to be found in an evidentiary sample (e.g., lidocaine, tributyoxyethyl phosphate [TBEP]), or it may contain substances that can also act as a positive control or reference material (e.g., sodium, ammonium, and potassium ions).

A positive control, also referred to as a reference material, known material, or standard, is a single substance or a mixture of substances which are of known origin and/or composition and are expected to be found in an evidentiary sample and can be used for comparison between the two samples (e.g., nitroglycerin [NG], trinitrotoluene [TNT], black powder). Positive controls may include laboratory grade chemicals, commercial products, and synthesized materials.

2 SCOPE

This procedure describes instrument parameters, method information, and operations and is to be used in conjunction with the instrument performance documents for individual instruments and the procedures for the analysis of evidence. This procedure applies to caseworking personnel conducting work in explosives chemistry.

3 EQUIPMENT

Equivalent equipment, materials, and reagents may be substituted as needed.

3.1 Equipment

- Chromatography columns (gas, liquid, ion)
- SEM stubs or carbon planchets with liquid adhesive (e.g., Duro-tak), carbon adhesive tabs, or aluminum or copper tape
- Solid phase microextraction (SPME) fibers
- General laboratory supplies

3.2 Instruments

- Fourier transform infrared (FTIR) spectrometer with attenuated total reflectance (ATR) or microscope attachment
- Gas chromatograph with electron capture detector (GC/ECD)
- Gas chromatograph with flame ionization detector (GC/FID)
- Gas chromatograph with mass spectrometer (GC/MS)
- Headspace gas chromatograph with mass spectrometer (HS-GC/MS)

- Ion chromatograph (IC)
- Liquid chromatograph with mass spectrometer (LC/MS)
- Microscope (optical or digital) with optional digital camera
- Raman spectrometer with macro compartment or microscope attachment
- Scanning electron microscope with energy dispersive X-ray spectrometer (SEM/EDS)
- Ultra performance liquid chromatograph with mass spectrometer (UPLC/MS)
- X-ray diffractometer (XRD)

3.3 Chemicals/Reagents

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- Other purchased reference and known materials for testmixes, standards, and positive controls

4 STANDARDS AND CONTROLS

Refer to the Explosives Quality Assurance and Operations Manual for details regarding verification of reference materials. Testmix components, preparation instructions, storage requirements (if applicable), and re-verification requirements are recorded in the applicable instrument performance document(s). Refer to the Explosives Level 4 Documents for guidance on preparing positive control solutions.

The positive control solutions described below do not expire but should be re-evaluated for continued use after two years unless otherwise noted.

4.1 Positive Controls

Refer to the explosives chemistry technical procedures for specific positive controls and standards that are relevant to the analysis being conducted. The following testmixes that are used for checking instrument performance are also used as positive controls for casework: GC/ECD, GC/FID, headspace GC/MS (volatiles testmix), IC, LC/MS (ESI and APCI), and UPLC/MS. Refer to the Fire Debris and Ignitable Liquid Analysis procedure for ignitable liquid reference material information.

Record stock solution preparations in the reagent log. Other preparations may be recorded in the examination records. PTFE-lined lids must be used with all standards made with hexane. The concentration of testmix or standard components may be modified to coincide with instrument response.

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The standard and intermediate stock solution will be maintained in colored or amber bottles in a refrigerator.

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5 INSTRUMENT PARAMETERS

The following instrument parameters are not intended to be prescriptive nor exhaustive. Minor modifications to the conditions may be used as needed, provided the same parameters (or similar parameters for some techniques, e.g. SEM/EDS) are used for all applicable solvent blanks, control samples, and questioned items; and the Positive Control(s) provide acceptable data. The utilized parameters will be recorded and retained with the case notes and/or data.

Mobile phase preparations (i.e., IC, LC, UPLC) may be scaled appropriately.

5.1 FTIR

Most FTIRs include an ATR accessory and may include focusing lenses such as ZnSe or KRS-5 to allow for different scan limits. Analyses should be performed in ATR mode whenever possible.

- Mode: Reflectance
- Number of scans: 32
- Resolution: 4
- Scan range: 400-4000 cm^{-1} or greater as allowed by the instrument

Clean the ATR sampling surface with methanol or another appropriate solvent before and after analyzing samples. Collect a background spectrum with the ATR accessory in the open position prior to or immediately after analyzing a sample.

5.2 GC/ECD

Method Name: EXPSPLIT (or equivalent)

- Inlet/Injector
  - Injection volume: 1.0 µL
  - Inlet: split
  - Split ratio: 5:1
  - Inlet temp: 225°C
  - Pressure: ~9.5 psi
- Oven
  - Initial temp: 50°C
  - Initial time: 1.5 min
  - Ramp: 25°C/min
  - Final temp: 250°C
  - Final time: 10 min
- Column
  - Type: J&W DB-5ms, 0.25 mm diameter, 0.25 µm film thickness, ~6 m length
  - Mode: Constant makeup flow
  - Initial pressure: 9.5 psi
  - Nominal initial flow: 3.7 mL/min
  - Carrier gas: Helium
- Detector
  - Temperature: 275°C
  - Makeup flow: 25 mL/min
  - Const column + makeup: ~28.7 mL/min

5.3 GC/FID

Method Name: HIGHTEMP (or equivalent)

- Inlet/Injector
  - Injection volume: 1.0 µL
  - Inlet: Splitless
  - Initial temp: 55°C
  - Ramp rate: 500°C/min
  - Ramp time: 10 min
  - Final temp: 400°C
- Oven
  - Initial temp: 55°C
  - Initial time: 2.0 min
  - Ramp 1: 30°C/min
  - Ramp 1 Time: 0 min
  - Final temp 1: 100°C
  - Ramp 2: 15°C/min
  - Final temp 2: 400°C
  - Ramp 2 time: 3.5 min
  - Run time: 27.0 min
  - Equil time: 0.5 min
- Column
  - Type: Zebron ZB-1HT, 0.25 mm diameter, 0.1 µm film thickness, 15 m length
  - Mode: Constant flow
  - Flow rate: 1.0 mL/min
  - Carrier gas: Helium
- Detector
  - Temperature: 420°C
  - Mode: Constant makeup flow
  - Hydrogen flow: 40.0 mL/min
  - Air flow: 450.0 mL/min
  - Makeup flow: 30 mL/min
  - Makeup gas: Nitrogen

5.4 GC/MS in Chemical Ionization (CI) Mode

Method Name: EXPLNICI (or equivalent)

- Inlet/Injector
  - Injection volume: 1.0 µL
  - Inlet: split
  - Split ratio: 10:1
  - Inlet temp: 200°C
- Column
  - Type: J&W DB-5MS, 0.25 mm diameter, 0.25 µm film thickness, ~30 m length
  - Mode: Constant makeup flow
  - Initial pressure: 9.5 psi
  - Nominal initial flow: 3.7 mL/min
  - Carrier gas: Helium

- Oven
  - Initial temp: 60°C
  - Initial time: 2 min
  - Ramp: 35°C/min
  - Final temp: 260°C
  - Final time: 2.3 min
  - Total run time: 10 min
- Reagent Gas
  - Methane (2.0 mL/min)
- Detector
  - Ionization: Negative ion mode
  - Scan range: 43-400 m/z
  - Solvent delay: 2.5 min

5.5 GC/MS in Electron Ionization (EI) Mode

- Column (used for all methods)
  - Type: J&W DB-5MS, 0.25 mm diameter, 0.25 µm film thickness, ~30 m length
  - Mode: Constant makeup flow
  - Initial pressure: 9.5 psi
  - Nominal initial flow: 3.7 mL/min
  - Carrier gas: Helium

Method name: EXPL (or equivalent)

- Inlet/Injector
  - Injection volume: 1.0 µL
  - Inlet: split
  - Split ratio: 10:1
  - Inlet temp: 250°C
- Oven
  - Initial temp: 60°C
  - Initial time: 3 min
  - Ramp: 30°C/min
  - Final temp: 260°C
  - Final time: 10 min
- Detector
  - Scan range: 43-400 m/z
  - Solvent delay: 3 min

Method name: SP (or equivalent)

- Inlet/Injector
  - Injection volume: 1.0 µL
  - Inlet: split
  - Split ratio: 10:1
  - Inlet temp: 170°C
- Oven
  - Initial temp: 45°C
  - Initial time: 3 min
  - Ramp: 15°C/min
  - Final temp: 150°C
  - Ramp 2: 40°C/min
  - Final temp 2: 265°C
  - Final time: 7 min
- Detector
  - Scan range: 41-400 m/z
  - Solvent delay: 3 min

Method name: PLASTIC (or equivalent)

- Inlet/Injector
  - Injection volume: 1.0 µL
  - Inlet: Splitless
  - Inlet temp: 250°C
- Oven
  - Initial temp: 60°C
  - Initial time: 2 min
  - Ramp: 50°C/min
  - Final temp: 280°C
  - Final time: 8 min
- Detector
  - Scan range: 40-400 m/z
  - Solvent delay: 3 min

Method name: SUGAR (or equivalent)

- Inlet/Injector
  - Injection volume: 1.0 µL
  - Inlet: splitless
  - Inlet temp: 250°C
- Oven
  - Initial temp: 75°C
  - Initial time: 0 min
  - Ramp: 35°C/min
  - Final temp: 180°C
  - Hold: 2 min
  - Ramp 2: 10°C/min
  - Temp 2: 215°C
  - Ramp 3: 15°C/min
  - Final temp: 285°C
  - Final time: 2 min
- Detector
  - Scan range: 50-275 m/z
  - Solvent delay: 5 min

5.6 Headspace GC/MS (EI)

The Headspace GC/MS can be used with a heated, gas-tight syringe or with a SPME fiber, ensuring that the compatible liner is used for each technique. SPME fibers should be

conditioned as needed using the automated bakeout option and needle heater, by manually inserting into the needle heater, or by manually inserting into the GC inlet at 250°C for 30 minutes.

- Column (used for all methods)
  - Type: J&W DB-624, 0.25 mm diameter, 1.4 µm film thickness, ~30 m length
  - Mode: Constant makeup flow
  - Initial pressure: 9.5 psi
  - Nominal initial flow: 3.7 mL/min
  - Carrier gas: Helium

Method name: Volatiles_Split_HS_10mL and Volatiles_Split_HS_20mL (or equivalent)	Method name: LightGases_HS_20mL (or equivalent)

• Incubation
  ○ Incubator: 80°C for 5 min
• Inlet/Injector
  ○ Injection volume: 1.0 mL from HS syringe at 90°C
  ○ Inlet: Split
  ○ Split ratio: 10:1
  ○ Inlet temp: 150°C
• Oven
  ○ Initial temp: 40°C
  ○ Initial time: 4 min
  ○ Ramp: 10°C/min
  ○ Final temp: 120°C
  ○ Ramp 2: 30°C/min
  ○ Final temp 2: 250°C
  ○ Total run time: 18 min
• Detector
  ○ Scan range: 29-400 m/z
	• Inlet/Injector
	  ○ Injection volume: 300 µL from HS syringe at 40°C
	  ○ Inlet: Split
	  ○ Split ratio: 50:1
	  ○ Inlet temp: 50°C
	• Oven
	  ○ Initial temp: 30°C
	  ○ Initial time: 1 min
	  ○ Ramp: 10°C/min
	  ○ Final temp: 50°C
	  ○ Final time: 4 min
	  ○ Total run time: 7 min
	• Detector
	  ○ Scan range: 20-150 m/z
	  ○ Solvent delay: 0 min

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• SPME Fiber
  ○ Type: Supelco StableFlex, 65 µm PDMS-DVB coating
• Incubation
  ○ Incubator: 60°C for 1 min
  ○ Extraction time: 5 min
  ○ Desorption time: 30 sec
• Fiber Bakeout
  ○ Pre bakeout time: 5 min at 250°C
• Inlet/Injector
  ○ Inlet: Splitless
  ○ Inlet temp: 150°C
• Oven
  ○ Initial temp: 60°C
  ○ Initial time: 0 min
  ○ Ramp: 20°C/min
  ○ Final temp: 240°C
  ○ Final time: 1 min
  ○ Total run time: 10 min
• Detector
  ○ Scan range: 29-400 m/z
  ○ Solvent delay: 3.5 min
	• SPME Fiber
	  ○ Type: Supelco StableFlex, 65 µm PDMS-DVB coating
	• Incubation
	  ○ Incubator: Not used
	  ○ Extraction time: 0 sec
	  ○ Desorption time: 30 sec
	• Fiber Bakeout
	  ○ Post bakeout time: 5 min at 250°C
	• Inlet/Injector
	  ○ Inlet: Splitless
	  ○ Inlet temp: 150°C
	• Oven
	  ○ Initial temp: 60°C
	  ○ Initial time: 0 min
	  ○ Ramp: 20°C/min
	  ○ Final temp: 240°C
	  ○ Final time: 1 min
	  ○ Total run time: 10 min
	• Detector
	  ○ Scan range: 29-400 m/z
	  ○ Solvent delay: 3.5 min

5.7 IC

Nitric Acid System
- Mobile phase: 3.0 mM HNO_3/0.1 mM EDTA
- Program: Isocratic
- Flow rate: 1.0 mL/min
- Column: Waters IC-Pak C M/D column
- Injection volume: 10 µL
- Acquire time: 15 min
- Detector: Conductivity Detector

Potassium Hydroxide Anions System
- Mobile phase: Potassium hydroxide
- Program: Gradient
  - 20 mM at 0 min, 30 mM at 9 min, 80 mM at 13 min, 20 mM at 21.2 min
- Column: Dionex IonPac AS19
- Injection volume: 25 µL
- Acquire time: 25 min
- Detector: Suppressed conductivity detector

Methanesulfonic Acid Cations System
- Mobile phase: Methanesulfonic acid
- Program: Isocratic
- Column: Dionex IonPac CS12A
- Injection volume: 25 µL
- Acquire time: 15 min
- Detector: Suppressed conductivity detector

Potassium Carbonate Anions System
- Mobile phase: Potassium carbonate
- Program: Isocratic
- Column: Dionex IonPac AS22
- Injection volume: 25 µL
- Acquire time: 16 min
- Detector: Suppressed conductivity detector

5.7.1 Mobile Phase Preparation for the Nitric Acid Cations Systems

The mobile phase for the nitric acid Cations Systems contains 3.0 mM HNO_3 with 0.1 mM EDTA in deionized water. To prepare, add 0.0292 g EDTA and 189 µL HNO_3 to a 1-L volumetric flask and dilute to volume with deionized water.

If preparing a larger stock solution that will be used over an extended period of time, the stock solution will be stored in a plastic container.

5.8 LC/MS

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Method name: EXP (or equivalent)
- Liquid Chromatograph
  - Mobile Phase: 60% methanol / 40% 3.125 mM ammonium nitrate in deionized water
  - Flow Rate: 0.3 mL/min
  - Column: C-18, 5 µm column, 150 mm length, 2.1 mm diameter
  - Injection volume: 5 µL
- Mass Spectrometer
  - Ionization: ESI
  - Tune file: Exp_Tune
  - Scan type: Full scan
  - Scan range: 200-400 m/z
  - Polarity: Negative
  - Data type: Centroid
  - Acquire time: 10 min
- Source Parameters
  - See validation file

- Liquid Chromatograph
  - Mobile Phase: Methanol with 1.25 mM ammonium nitrate / deionized water with 1.25 mM ammonium nitrate
  - Flow Rate: 0.3 mL/min
  - Program: Gradient
    - 90% Water at 0-2 min, 50% Water at 12-14min, 90% Water at 17-20 min
  - Column: C-18, 5 µm column, 150 mm length, 2.1 mm diameter
  - Injection volume: 10 µL
- Mass Spectrometer
  - Ionization: APCI
  - Redacted
  - Scan type: Full scan
  - Scan range: 150-250 m/z
  - Polarity: Positive
  - Data type: Centroid
  - Acquire time: 12 min
- Source Parameters
  - See validation file

Run/acquire time and mass scan range may be adjusted as necessary to identify additional analytes.

5.8.1 Mobile Phase Preparation for EXP Method

The mobile phase consists of the following two components contained within separate reservoirs in the LC system:

A. 3.125 mM ammonium nitrate in deionized water. To prepare, add 0.250 g of ammonium nitrate to a 1-L volumetric flask and dilute to volume with deionized water.

B. 100% Methanol.

5.8.2 Mobile Phase Preparation for Redacted

The mobile phase consists of the following two components contained within separate reservoirs in the LC system:

A. 1.25 mM ammonium nitrate in methanol. To prepare, add 0.100 g of ammonium nitrate to a 1-L volumetric flask and dilute to volume with methanol.

B. 1.25 mM ammonium nitrate in deionized water. To prepare, add 0.100 g of ammonium nitrate to a 1-L volumetric flask and dilute to volume with deionized water.

5.9 Microscopes (Digital and Optical) with Digital Camera

Microscopes with digital cameras and measuring capabilities will be verified (every magnification level) using a ruler or stage micrometer after each annual maintenance service.

5.10 Raman Spectroscopy

Samples for Raman analysis may be analyzed either in a sample compartment or through a microscope attachment using one or more of the following laser wavelengths: 785 nm, 780 nm, 532 nm.

Thermo DXR

- Collect Tab
  - Exposure time: 20 sec
  - Number of exposures: 2
  - Final format: Shifted spectrum (cm^{-1})
  - Cosmic ray threshold: Medium
  - Correction: None
  - Background exposures: 2

- Bench Tab
  - Beam path/accessory: Microscope or 180-Degree
  - Laser power: 24 mW (0-24 mW available)
  - Focus: Set to achieve maximum signal
  - Aperture: 50 µm pinhole (25 µm and slit options available)
  - Objective: 10x, microscope only
  - Resolution: High
  - Grating: 400 lines/mm
  - Range: 200-3300 cm^{-1}

5.11 SEM/EDS

- Live time: 100 sec (or longer)

- Amp time: 6.4 µs (or appropriate setting)
- Voltage: 25 keV (or appropriate depending on sample)
- Working distance and beam intensity/spot size will be set at the individual's discretion

The backscatter detector may be used to visualize elemental contrast for imaging or for locating an area to analyze by EDS.

For image collection, values for accelerating (high) voltage, working distance, spot size, beam intensity, stigmation, focus, brightness, and contrast are established at the individual's discretion based on image quality desired.

Trace quantities of elements may require an acquisition time of 500 live seconds or longer to achieve the desired signal to noise ratio (SNR). Decreasing the Amp Time and increasing the beam intensity can be used to increase the count rate and improve the SNR so long as it does not cause sample damage.

Appropriate sample holders include stubs made of aluminum, copper, or brass, a carbon planchet, or sample clamp. Adhesive carbon tabs or liquid adhesives (e.g., Duro-tak) can be used to adhere the sample to the holder. Metallic tapes, such as aluminum and copper tape, may also be used depending upon the analytes of interest.

5.12 UPLC/MS

Method name: EXP (or equivalent)

- Liquid Chromatograph
  - Mobile phase: 60% A1, 40% B1, Isocratic
  - Total flow: 0.5 mL/min
  - Column: C-18, 50 mm length, 2.1 mm internal diameter, 1.6 µm particle size
  - Column temp: 30° C
  - Guard column: 5 mm length, 2.1 mm internal diameter, 1.6 µm particle size
  - Injection volume: 8 µL
  - Total time: 2.0 min
- Autosampler Parameters
  - Autosampler temp: 14°C
  - Weak wash volume: 600 µL
  - Strong wash volume: 200 µL
  - Cycle Inject Valve: At 1 min
- Mass Spectrometer
  - Ionization: ESI
  - Scan type: Full scan
  - Runtime: 0 to 2 min
  - Polarity: Negative
  - Resolution: 17,500
  - Tune file: ESI_EXP_Neg
  - Scan range: 200-400 m/z
  - Data type: Profile
- Source Parameters
  - See validation file

A fresh blank consisting of 50:50 methanol:deionized water should be used between samples.

Run/acquire time and mass scan range may be adjusted as necessary to identify additional analytes.

5.12.1 Mobile Phase Preparation for EXP Method

UPLC systems are vulnerable to clogs due to microbial growth. Mobile phase components should be changed regularly (follow chart below), and associated glassware should be cleaned regularly to minimize this risk.

Mobile Phase Composition		
Component	**Composition**	**Replacement Schedule**
A1	100% Methanol	Replace when low.
B1	3.125 mM Ammonium Nitrate in Deionized Water	Prepare in small quantities. Replace after 4 weeks.
Weak Wash	50:50 Methanol:Deionized Water	Prepare in small quantities. Replace after 4 weeks.
Strong Wash	90:10 Methanol:Deionized Water	Prepare in small quantities. Replace after 4 weeks.
Seal Wash	50:50 Methanol:Deionized Water	Prepare in small quantities. Replace after 4 weeks.

The B1 mobile phase is prepared by adding 0.250 g of ammonium nitrate to a 1-L volumetric flask (or 0.125g to a 500-mL volumetric flask) and diluting to volume with deionized water.

5.13 XRD

Rigaku MiniFlex (or equivalent)

- X-ray generator: 40 kV, 15 mA
- Detector: D/teX Ultra
- Scan mode: Continuous
- Scan speed: 20 deg/min
- Step width: 0.02 deg
- Scan range: 5-80 deg
- Sample spin: Enabled

Appropriate sample holders include zero background holders with or without a depression.

6 LIMITATIONS

Refer to the Instrument Decision Criteria for Explosives Chemistry Analysis procedure for details regarding the acceptance of data generated using the instruments and methods described above.

7 REVISION HISTORY

Revision	Issued	Changes
09	09/30/2022	Updated to new document template. Removed testmix sections. Removed positive control preparation instructions and added reference to level 4 document. Removed solids probe. Updated IC methods in 5.7 to method type instead of instrument manufacturer. Added new SUGAR method for GC/MS (EI). Added reference to new Instrument Decision Criteria procedure in Limitations. Added ASTM references.
10	04/06/2023	Updated sections 4.1.4 and 4.1.5.