

General Instrument Maintenance Protocol

1 Scope

The purpose of this document is to provide definitions and general guidelines for the interpretation of the specific performance monitoring protocol available for each type of instrument. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, paint, explosives (chemistry), fire debris, and Chemistry Unit general physical and chemical analysis.

2 Principle

Instruments available for the analysis of evidence are purchased from a variety of different manufacturers. All instruments eventually require maintenance, troubleshooting, and repair. Although the user interface and hardware fittings may differ, the overall instrument principles and maintenance are consistent.

This protocol divides instrument maintenance into two categories: Preventative and corrective. Preventative maintenance involves routine monitoring of performance, adjustment of common parameters (e.g., head pressure, solvent degas), and replacement of consumable items (e.g., septa, columns) in order to ensure reproducible and uninterrupted operation. Corrective maintenance may be required when poor performance is observed or the instrument fails to operate properly.

All performance monitoring protocols are based upon manufacturer's recommendations. Users are encouraged to refer to the manufacturer's instrument manuals for more information on maintenance and troubleshooting. Users will be familiar with the operation of the instrument as described in the manual(s), specific instrument performance monitoring protocols, appropriate discipline SOPs and receive training from instrument support personnel, a trained operator, and/or the instrument manufacturer before operating such equipment.

The maintenance and operating procedures are categorized by how often they will be performed (daily, monthly, and/or as needed) to insure the integrity of the system. These terms are approximate time intervals, based on instrument use, and allow for weekends and other periods of instrument inactivity. The term 'daily' refers to each day the instrument is used for analysis. The term 'monthly' refers to each calendar month, not to exceed 45 calendar days from the previous month's date of maintenance. The term 'as needed' refers to maintenance that is to be performed based on system performance or major interruptions in service. If other intervals will be followed, they will be specified in the applicable SOP.

3 Equipment/Materials/Reagents

Any materials (such as pump oil and solvents) and all replacement parts will meet manufacturer's specifications and recommendations. Manufacturer's instrument manuals and specific performance monitoring protocols are generally the best source for this information. Note that performance monitoring protocols refer to the manufacturer's name at the time of installation. Refer to the appropriate instrument support personnel for updated contact information for instrument parts, documentation, and service.

4 Standards and Controls

All standards, solutions, and mobile phases required are specified in the appropriate SOP.

5 Calibration

Any procedures used to calibrate and/or verify the integrity of the instrument will be specified in the appropriate SOP. Instruments that are calibrated by an outside vendor, such as pipettes and micrometers, are tracked in the Forensic Advantage (FA) Resource Manager (RM).

6 Sampling or Sample Selection

Not applicable.

7 Abbreviations and Definitions

- a. SOP - Standard Operating Procedure. Interchangeably used in place of Performance Monitoring Protocol.
- b. QA/QC - Quality Assurance/Quality Control
- c. IOSS - Instrument Operation and Systems Support
- d. CU - Chemistry Unit
- e. Daily, Monthly, Yearly, As Needed - refer to Principle section
- f. Manufacturer's Instrument Manual(s) - paper or electronic instrument documentation provided by the manufacturer.
- g. Tuning - adjusting parameters (e.g., lens voltages) to maximize instrument performance
- h. Calibration - correcting instrument responses to a known value (e.g., mass correction performed on a Time-of-Flight mass spectrometer).
- i. GC - Gas Chromatograph(y). Refer to the "Gas Chromatograph General Maintenance Protocol" for GC-specific maintenance, and troubleshooting.
- j. LC - Liquid Chromatograph(y). Refer to the "Liquid Chromatograph General Maintenance Protocol" for LC-specific maintenance, and troubleshooting.
- k. HPLC - High Performance Liquid Chromatography (used synonymously with LC above).
- l. MS - Mass Spectrometer (Spectrometry). Refer to the "Mass Spectrometer General

Maintenance Protocol" for MS-specific abbreviations, theory, maintenance, and troubleshooting.

- m. TOF - Time-of-Flight (Mass Spectrometer, Spectrometry)
- n. FTIR - Fourier Transform Infrared (Spectrophotometer, Spectrophotometry)
- o. ATR - Attenuated Total Reflectance (FTIR Accessory, Objective)
- p. UV-Vis - Ultraviolet-Visible (Light Source, Spectrophotometer)
- q. SNR - Signal to Noise Ratio (SNR). A comparison of the electronic response of an analyte to the baseline noise.
- r. Peak - A detector response that rises above the observed baseline. A response is considered a peak if it has a minimum SNR of 3:1.
- s. Chromatogram – the detector response chart generated by a chromatographic instrument, generally plotted as response versus time.
- t. Performance Standard/Testmix – a standard, known chemical or mixture of chemicals used to test the performance of an instrument.
- u. Operator - a chemist trained to use the instrumentation.
- v. NIST - National Institute of Standards and Technology
- w. TIC - Total Ion Chromatogram
- x. RIC - Reconstructed Ion Chromatogram
- y. EI - Electron Impact (Ionization)
- z. Profile/Continuum - Mass spectrometer data collected continuously without centroiding
- aa. Centroid - Centered, non-continuous mass spectrometer data.
- bb. m/z - Mass-to-Charge Ratio
- cc. Unit-Mass - refers to the mass resolution of a standard quadrupole or ion trap mass spectrometer
- dd. Accurate Mass - refers to the mass accuracy of a high-resolution mass spectrometer such as a Time-of-Flight (TOF)
- ee. MCP - Micro Channel Plate
- ff. RMS - Root Mean Square
- gg. RSD - Relative Standard Deviation

8 Procedures

8.1 Performance Monitoring

The purpose of the performance monitoring protocols is to verify and track reproducibility, quality, accuracy, and reliability of instrument operation and generated data from analysis to analysis, day to day, and year to year. This includes recording specific instrument parameters and performing and recording specific tasks. This information is then available to track instrument performance patterns or to be used in court. These tasks are outlined under the 'Procedures' section of the performance monitoring protocols.

8.2 Preventative Maintenance

In order to prevent instrument downtime and casework delays, certain maintenance tasks will be required to be performed on a routine, predetermined schedule - daily, monthly, or yearly. These tasks will usually involve replacing parts before they cause problems. They are outlined under the 'Procedures' section of the performance monitoring protocols.

Each type and model of an instrument may have different, specialized components requiring specific preventative maintenance. Suggested step-by-step directions for specific maintenance procedures may be found in the manufacturer's instrument manuals. When performed, all preventative maintenance will be entered into the appropriate QA/QC log.

8.3 Corrective Maintenance

Evidence of poor performance or instrument malfunction should indicate to the operator to take corrective measures. There are some things the operator may try before contacting appropriate instrument support personnel to resolve the issue, depending on their level of training and comfort. Tips on general troubleshooting are provided by appropriate instrument support personnel, and can be found in the main instrument room. In addition, the operator may consult the manufacturer's instrument manual. If the operator is still unable to correct the problem, they can contact appropriate instrument support personnel by submitting a request for repair. All corrective maintenance will be entered into the appropriate QA/QC log.

8.3.1 Poor Performance

The necessity for maintenance will occur when the instrument fails to meet protocol decision criteria specifications or if other poor performance, such as a loss of sensitivity, is observed. Follow the above requirement in 8.3.

8.3.2 Instrument Malfunction

In the event of an instrument malfunction such as hardware or software failure that cannot be resolved by the above requirement in 8.3, appropriate instrument support personnel will contact the instrument manufacturer's service representative.

8.4 Records

Any instrument logsheets and logbooks referred to in the 'Procedures' section of each SOP can be either paper or electronic format. Any example QA/QC logs and printouts are for reference only and may differ in appearance and form from the actual records generated.

- a. All instruments that have a series of performance checks (such as daily or monthly) will have a QA/QC log. The operator will enter the appropriate information required by the SOP 'Procedures' section.
- b. Upon completion and passing of all checks, the operator will print the necessary

reports and initial each page. If multiple pages are stapled together, only the first page needs to be initialed. The printout(s) will be placed in the three-ring QA/QC binder in the appropriate section(s).

- c. The operator will record sample types, problems, pass/fail, maintenance, and comments in the QA/QC log, as appropriate.

9 Instrumental Conditions

Any parameters required to monitor the performance of an instrument will be specified in the appropriate SOP.

9.1 Minor Modifications

Some of the instrumental conditions referenced in the 'Instrument Conditions' section of an SOP may be slightly modified to obtain optimum instrument performance on a specific instrument. Any minor modifications to a performance monitoring protocol will require the approval of the IOSS Manager or appropriate instrument support personnel. The modification and its approval will be recorded in the instrument QA/QC log.

10 Decision Criteria

Every performance monitoring protocol will have specific decision criteria to determine if the instrument is operating properly. If these should fail, refer to the 'Corrective Maintenance' section of this protocol in conjunction with the instrument-specific SOP.

11 Calculations

Not applicable.

12 Measurement Uncertainty

Not applicable.

13 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument. Instrument-specific limitations will be specified in the appropriate SOP.

14 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

15 References

Instrument Operation and Systems Support SOP Manual.

Manufacturer's Instrument Manuals for the specific models and accessories used.

"Gas Chromatograph General Maintenance Protocol" (Inst 002) *Instrument Operation and System Support SOP Manual.*

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and Systems Support SOP Manual.*

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual.*

FBI Laboratory Safety Manual.

FBI Laboratory Quality Assurance Manual.

FBI Laboratory Operations Manual.

Rev. #	Issue Date	History
0	06/21/06	New document that replaces original which was titled "General Instrument Maintenance Protocol."
1	05/01/08	Updated 'daily' and 'monthly' descriptions in Section 2, and removed reference to green logbooks in Section 8.4c. Added information regarding calibration by outside vendors to Section 5. Corrected numbering error in Sections 14 and 15.
2	10/04/18	Changed title from 'Policy' to 'Protocol'. Updated Section 1 Scope to include applicable disciplines/categories of testing. Added FA RM to Section 5. Updated heading in Section 6. Updated abbreviation for IOSS in Sections 7, 15, and header. Changed IOSS to 'appropriate instrument support personnel' in Sections 3, 8.3, 8.3.2 and 9.1. Clarified stapling of pages in Section 8.4 b. Changed Section 9.1 to 'minor modifications' to a performance monitoring protocols' for clarity.

Approval

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Date: 09/28/2018

Gas Chromatograph General Maintenance Protocol

1 Scope

The purpose of this protocol is to provide general guidelines for maintenance of gas chromatography instruments. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, paint, explosives (chemistry), fire debris, and Chemistry Unit general physical and chemical analysis. Refer to the "General Instrument Maintenance Protocol" for overall instrument maintenance guidelines and definitions.

2 Principle

Gas chromatography (GC) instruments available for the analysis of evidence are purchased from a variety of different manufacturers. All instruments eventually require maintenance, troubleshooting, and repair. Although the user interface and hardware fittings may differ, the overall instrument principles and maintenance are similar. Information and procedures concerning performance monitoring of a specific GC can be found in the corresponding instrument's performance monitoring protocol.

3 Equipment/Materials/Reagents

Any materials (e.g., septa, columns, liners) and all replacement parts will meet the manufacturer's specifications and recommendations. Manufacturer's instrument manuals and specific performance monitoring protocols are generally the best source for this information.

4 Standards and Controls

All standards, solutions, and carrier gases are specified in the appropriate SOP.

5 Calibration

Any procedures used to calibrate and/or verify the integrity of the instrument will be specified in the appropriate SOP.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Preventative Maintenance

Each type and model of an instrument may have different, specialized components requiring specific preventative maintenance. Suggested step-by-step directions for specific maintenance procedures may be found in the corresponding manufacturer's instrument manuals for individual instruments. When performed, all preventative maintenance will be entered into the appropriate QA/QC log. The following procedures are generic in nature and are included for reference. Daily, monthly, and/or as needed checks are outlined in the appropriate performance monitoring protocol.

7.1.1 Injector

The injector is the most likely place that unwanted sample residue and analytical artifacts may collect over periods of usage. Regular replacements of the septum and liner will aid in reduction and removal of these undesirable interferences. The intervals for checking and replacement are provided in the performance monitoring protocol and QA/QC log for individual systems. Internal injector parts should not be touched with bare hands. It is recommended that lint-free gloves be worn when needed and that the operator cool heated areas on the instrument prior to any maintenance.

7.1.1.1 Septum and Liner Replacement

- a. Set the oven temperature to 30°C.
- b. Turn off the detector. If the detector is a mass spectrometer, it can stay on.
- c. Cool the inlet to room temperature and turn off the inlet pressure.
- d. Using the manufacturer's instrument manuals as a reference, remove the septum and liner retainer nut. Remove the old septum with tweezers and replace it with a new septum.
- e. Remove the old liner from the injector with tweezers.
- f. Place an O-ring on the new liner about 5 to 7 mm from its top end.
- g. Place the liner straight down into the inlet and replace the septum and liner retainer nut.
- h. Restore the instrument conditions.

7.1.1.2 Replacing the Inlet Base Seal

- a. Cool the oven to room temperature and then turn the oven off.
- b. Turn off the detector. If the detector is a mass spectrometer, it can stay on.
- c. Cool the inlet to room temperature and turn off the inlet pressure.
- d. Using the manufacturer's instrument manuals as a reference, remove the column from the inlet. Cap the open end of the column with a septum or other suitable material to prevent contamination.
- e. Use a wrench to loosen the reducing nut and remove it. Remove the washer and seal inside the reducing nut.
- f. Replace the inlet base seal and washer in the reducing nut.
- g. Replace the reducing nut and tighten using a wrench.
- h. Reinstall the column following steps 7.2 c – i.

7.2 Corrective Maintenance

7.2.1 Column

Typically, the overall separation performance of a GC column will degrade over time, requiring corrective maintenance. Column maintenance is performed as needed based on instrument performance. The column ends should not be touch with bare hands. It is recommended that lint-free gloves be worn when possible and that the operator cool heated areas on the instrument prior to any maintenance.

7.2.1.1 Clipping the Column

- a. Set inlet and oven to room temperature.
- b. Turn off the detector. If the detector is a mass spectrometer, it can stay on.
- c. Remove the column from the inlet and remove the column nut from the column.
- d. Place the column nut and a new ferrule on the injector end of the column.
- e. Score the column using a column cutter. The score must be square to ensure a clean break.
- f. Break off the column end and inspect with a magnifying glass to ensure there are no burrs or jagged edges.

- g. Position the column so it extends the required length above the end of the ferrule as specified by the manufacturer. Mark the column underneath the column nut with a marker or typewriter correction fluid.
- h. Insert the column in the inlet and slide the nut and ferrule up the column to the inlet base. Finger tight the column nut, adjusting the column position so that the marker or correction fluid mark on the column is even with the bottom of the column nut.
- i. Tighten the column nut an additional $\frac{1}{4}$ to $\frac{1}{2}$ turn so that the column cannot be pulled out from the fitting.

7.2.1.2 Replacing the Column

- a. Turn off the detector. If detector is a mass spectrometer, it needs to be vented.
- b. Set inlet and oven to room temperature.
- c. After all heating zones are at room temperature, remove the column from the inlet and detector.
- d. Place a capillary column nut and ferrule on the injector end of the column.
- f. Score the column using a column cutter. The score must be square to ensure a clean break.
- g. Break off the column end and inspect with a magnifying glass to ensure there are no burrs or jagged edges.
- h. Position the column so it extends the required length above the end of the ferrule as specified by the manufacturer. Mark the column underneath the column nut with a marker or typewriter correction fluid.
- i. Insert the column in the inlet and slide the nut and ferrule up the column to the inlet base. Finger tight the column nut, adjusting the column position so that the marker or correction fluid mark on the column is even with the bottom of the column nut.
- j. Tighten the column nut an additional $\frac{1}{4}$ to $\frac{1}{2}$ turn so that the column cannot be pulled out from the fitting.
- k. The length of the column installed in the detector varies with detector type. Refer to the "Mass Spectrometer General Maintenance Protocol" and the manufacturer's instrument manuals for additional information.
- l. After column is installed at both the inlet and detector, establish a flow of the carrier gas and operating conditions. Most new columns do not require additional

conditioning. Refer to the materials provided with the column for specific information.

8 Instrumental Conditions

Refer to the appropriate procedures outlined in section 7, manufacturer's instrument manuals, or the instrument's performance monitoring protocol for specific instrumental conditions to be used during maintenance procedures.

9 Decision Criteria

Every performance monitoring protocol will have specific decision criteria to determine if the instrument is operating properly. If these should fail, the operator should refer to the 'Decision Criteria' section of the specific performance monitoring protocol. Additional information is provided in the 'Corrective Maintenance' section of the "General Instrument Maintenance Protocol".

10 Calculations

Not applicable.

11 Measurement Uncertainty

Not applicable.

12 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of any instrument. Instrument-specific limitations will be specified in the appropriate SOP.

13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

14 References

Instrument Operation and Systems Support SOP Manual

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

FBI Laboratory Quality Assurance Manual.

FBI Laboratory Operations Manual.

Rev. #	Issue Date	History
0	06/21/06	New document which replaces original also titled "Gas Chromatograph General Maintenance Protocol."
1	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Removed Chemistry Unit from Section 2. Updated abbreviation for IOSS in Section 14 and header. Updated section heading for Section 6 and updated wording in Section 9 for clarification.

Approval

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QA Approval

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Date: 09/28/2018

Liquid Chromatograph General Maintenance Protocol

1 Scope

The purpose of this protocol is to provide general guidelines for maintenance of liquid chromatography (LC) instruments. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, paint, explosives (chemistry), fire debris, and Chemistry Unit general physical and chemical analysis. Refer to the "General Instrument Maintenance Protocol" for overall instrument maintenance guidelines and definitions.

2 Principle

LC instruments available for the analysis of evidence are purchased from a variety of manufacturers. All instruments eventually require maintenance, troubleshooting, and repair. Although the user interface and hardware fittings may differ, the overall instrument principles and maintenance are similar. Refer to the "Mass Spectrometry General Maintenance Protocol" for mass spectrometry system maintenance. Information and procedures concerning performance monitoring of a specific LC can be found in the corresponding instrument's performance monitoring protocol.

3 Equipment/Materials/Reagents

Any materials (such as columns and filters) and all replacement parts will meet the manufacturer's specifications and recommendations. Manufacturer's instrument manuals and specific performance monitoring protocols are generally the best source for this information.

4 Standards and Controls

All standards, solutions, and mobile phases required are specified in the appropriate SOP.

5 Calibration

Any procedures used to verify the integrity of the instrument will be specified in the appropriate SOP.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Preventative Maintenance

Each type and model of an instrument may have different, specialized components requiring specific preventative maintenance. Suggested step-by-step directions for specific maintenance procedures can be found in the corresponding operations manuals for individual instruments. When performed, all preventative maintenance will be entered into the appropriate QA/QC log. The following procedures are generic in nature and are included for reference. Daily, monthly, and as needed checks are outlined in the appropriate performance monitoring protocol.

7.1.1 Mobile Phase Filtration

If using high quality High Performance Liquid Chromatography (HPLC) grade reagents, filtering mobile phases is not always necessary. However, filtration is highly recommended, particularly if a buffer is added.

7.1.2 Mobile Phase Degassing

The mobile phase should be degassed prior to entering the LC system in order to remove any dissolved oxygen and carbon dioxide. If the LC does not have this capability, degassing may be performed using a helium sparge or sonicator.

7.1.3 Pump Priming

Perform a 'Wet Prime' prior to connecting the column. Perform a 'Dry Prime' if any of the pump channels are dry.

7.1.4 Pump Cleaning

After use, the entire LC system should be flushed with an organic solvent such as methanol (MeOH) or isopropyl alcohol (IPA). The system should remain in the organic solvent until it is needed again for operation. When buffers are used, the system should be flushed with 100% water for a minimum of ten minutes prior to the organic solvent flush.

7.1.5 Column Maintenance

- a. Prior to use, the column must be equilibrated.
- b. After use, the column should be flushed and stored under appropriate conditions.
- c. Guard columns should be used to increase column lifetime. The frit inside should be periodically cleaned or replaced.

7.2 Corrective Maintenance

Each type and model of LC may have different, specialized components requiring specific corrective maintenance. Suggested step-by-step directions for specific procedures may be found in the corresponding manufacturer's instrument manuals for individual instruments. When performed, relevant corrective maintenance will be entered into the appropriate QA/QC logs. The following procedures are more complex options that may be pursued when troubleshooting the LC. The following steps are generic in nature and are included for reference.

7.2.1 Check Valves

The check valves can become dirty and degrade performance over time. They will be replaced as needed. To replace the check valves:

- a. Submerge the check valves in mobile phase or MeOH for several minutes prior to installation.
- b. Loosen and remove the tubing that connects to the check valve housing.
- c. Loosen and remove the check valve housing.
- d. Remove the check valve, noting the orientation in the housing.
- e. Place the new check valve in the housing using the proper orientation.
- f. Place the check valve housing back in the pump. Warning: Do not over-tighten check valve housing.
- g. Reconnect and tighten the tubing that connects to the check valve housing.
- h. Repeat for each check valve.

7.2.2 Pump Seals

The pump seals can deteriorate over time, causing inconsistent or poor pump operation. They should be replaced as needed. To replace pump seals:

- a. Remove all tubing and fittings connected to the pump head.
- b. Loosen the pump head retaining bolts evenly until the head can be removed from the pump.
- c. Remove the rod and fittings to expose the pump seal.
- d. Remove the old pump seal and clear any debris. The area can be washed with MeOH or IPA if necessary.

- e. Replace the rod and fittings.
- f. Place the pump head back in the pump and tighten the retaining bolts.
- g. Reconnect all tubing and fittings to the pump seal.
- h. Repeat for each pump head.

8 Instrumental Conditions

Refer to the appropriate instrument's performance monitoring protocol or manufacturer's instrument manuals for specific instrumental conditions to be used during maintenance procedures.

9 Decision Criteria

Every performance monitoring protocol will have specific decision criteria to determine if the instrument is operating properly. If these should fail, the operator should refer to the 'Decision Criteria' section of the specific performance monitoring protocol. Additional information is provided in the 'Corrective Maintenance' section of the "General Instrument Maintenance Protocol".

10 Calculations

Not applicable.

11 Measurement Uncertainty

Not applicable.

12 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of any instrument. Instrument-specific limitations will be specified in the appropriate SOP.

13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

14 References

Instrument Operation and Systems Support SOP Manual.

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual.*

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual.*

FBI Laboratory Safety Manual.

FBI Laboratory Quality Assurance Manual.

FBI Laboratory Operations Manual.

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0	06/21/06	New document which replaces original also titled "Liquid Chromatograph General Maintenance Protocol."
1	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Updated abbreviation for IOSS in Section 14 and header. Updated section heading for Section 6 and updated wording in Section 9 for clarification.

Approval

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IOSS Manager:

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Chemistry Unit Chief:

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QA Approval

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Mass Spectrometer General Maintenance Protocol

1 Scope

The purpose of this protocol is to provide general guidelines for maintenance of mass spectrometry (MS) instruments. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, paint, explosives (chemistry), fire debris, and Chemistry Unit general physical and chemical analysis. Refer to the "General Instrument Maintenance Protocol" for overall instrument maintenance guidelines and definitions.

2 Principle

These instruments are obtained from several different manufacturers. All instruments eventually require maintenance, troubleshooting, and repair. Although the user interface and hardware fittings may differ, the overall instrument principles and maintenance are similar. The mass spectrometers are typically used in tandem with either a gas chromatograph (GC) or a liquid chromatograph (LC). Refer to the "Gas Chromatograph General Maintenance Protocol" and the "Liquid Chromatograph General Maintenance Protocol" for chromatographic system maintenance.

3 Equipment/Materials/Reagents

Any materials (such as pump oil and solvents) and all replacement parts will meet manufacturer's specifications and recommendations. Manufacturer's instrument manuals and specific performance monitoring protocols are generally the best source for this information.

4 Standards and Controls

All standards, solutions, and gases required are specified in the appropriate SOP.

5 Tuning

As defined in the "General Instrument Maintenance Protocol" tuning refers to the adjusting of parameters (e.g., lens voltages) to maximize instrument performance. All mass spectrometers provide a general automatic tune function. Individual instrument performance monitoring protocols have specific information on use of this function. Mass spectrometers also provide the ability to manually tune the same parameters. Manual tuning can be used as needed, provided that the required documentation is provided, and the decision criteria specified in the performance monitoring protocol is achieved.

6 Calibration

Any procedures used to calibrate and/or verify the integrity of the instrument will be specified in the appropriate SOP.

7 Sampling or Sample Selection

Not applicable.

8 Procedures

8.1 Preventative Maintenance

Each type and model of an instrument may have different, specialized components requiring specific preventative maintenance. Suggested step-by-step directions for specific maintenance procedures may be found in the manufacturer's instrument manuals. The following procedures are generic in nature and are included for reference.

8.1.1 Ion Volume Cleaning

Systems equipped with a removable ion volume should have the volume cleaned regularly. Lint-free gloves should be worn during the disassembly and reassembly of the mass spectrometer. Volume performance can be monitored by use of the performance monitoring standard criteria outlined in the instrument performance monitoring protocol. Although the interval is left to the operator, it is suggested that the volume at least be visually inspected on a daily basis. Volume-cleaning is the responsibility of all MS-trained operators.

- a. Remove the inner ion volume (with the filament and column inlet holes) from the outer housing.
- b. Mix a slurry of aluminum oxide and methanol.
- c. Thoroughly clean the inner and outer surfaces of both pieces of the volume with the slurry. Using a cotton-tipped applicator, clean all dark or discolored areas, particularly around holes.
- d. Place the ion volume parts in a beaker with deionized water and sonicate for one minute.
- e. Thoroughly rinse the parts with deionized water followed by methanol.
- f. Re-assemble the volume.

8.1.2 Vacuum Pumps

All MS systems have one or more rough/mechanical pumps. It is suggested that the pump oil level and clarity be checked yearly, and changed if needed. Some systems also have one or more turbo pumps as well. Turbo pumps are very sensitive and vary greatly, even within the same instrument. It is suggested that the oil not be replaced in turbo pumps. For changing the oil in the rough/mechanical pumps:

- a. Vent the MS.
- b. Allow the pump to cool for at least 10 minutes before continuing.
- c. Open the pump vent/fill hole.
- d. Place a sturdy plastic container under the oil drain.
- e. Open the oil drain and allow the old oil to empty.
- f. Add 10-20 mL of fresh oil to the pump with the drain open in order to flush the system.
- g. Replace the cover on the oil drain.
- h. Fill the pump with fresh oil until the proper fill level is noted in the level indicator.
- i. Replace the cover on the pump vent/fill hole.
- j. Seal, label and dispose of the used oil as outlined in the *FBI Laboratory Safety Manual*.
- k. Repeat for each vacuum pump on the system.

8.2 Corrective Maintenance

Each type and model of an instrument may have different, specialized components requiring specific corrective maintenance. Suggested step-by-step directions for specific procedures may be found in the corresponding manufacturer's instrument manuals. When performed, relevant corrective maintenance will be entered into the appropriate QA/QC logs. The following procedures are generic in nature and are included for reference.

8.2.1 Source Bake-Out

It should be noted that an occasional overnight baking-out of the detector may be useful when elevated baselines and other interferences are observed. The source temperature can be temporarily raised to 250°C. The transfer line should not be set to a temperature above the limit of the GC column. Please refer to specific column documentation for more information.

- a. Note the current source temperature.
- b. Set the source temperature to 250°C.
- c. Allow the source to bake-out for several hours.
- d. Return the source temperature to the original setting.
- e. Allow the source to cool to the original temperature before operating the instrument.
- f. This procedure can be repeated keeping the source temperature elevated overnight. However, if the problems persist, it is likely that the source and/or analyzer need to be cleaned.

8.2.2 Source Cleaning

The source will be cleaned on MS systems as needed, based on system performance. All systems require the removal of the entire source.

- a. Vent the MS system and turn off the main power.
- b. Allow the source to cool before continuing.
- c. Open the vacuum manifold.
- d. Disconnect any gas lines or electrical connections to the source.
- e. Loosen and/or remove source retaining bolts and clips.
- f. Remove the source.
- g. Disassemble the source in order to separate the lenses and any surfaces that come in contact with the ionization chamber.
- h. Mix a slurry of aluminum oxide and methanol.
- i. Thoroughly clean the pieces of the source with the slurry. Using a cotton-tipped applicator, clean all dark or discolored areas, particularly around holes. Warning: Only clean metal surfaces.
- j. Place the parts in a beaker with deionized water and sonicate for one minute.
- k. Thoroughly rinse the parts with deionized water followed by methanol.
- l. Re-assemble the source.

- m. Place the source in the manifold and secure.
- n. Reconnect all gas lines and electrical connections.
- o. Seal the manifold.
- p. Turn on the main power and pump down the system, observing for vacuum leaks.

8.2.3 Analyzer Cleaning

MS systems in the Quantico laboratory employ three types of analyzers: Ion trap, quadrupole, and time-of-flight. In general, these analyzers do not need regular cleaning. However, an ion trap can be easily cleaned when venting the system for source cleaning. A quadrupole is more sensitive to shock and manipulation, and should only be cleaned when warranted by poor performance. If the time-of-flight needs to be cleaned, contact appropriate instrument support personnel.

8.2.3.1 Cleaning an Ion Trap

- a. Vent the MS system and turn off the main power.
- b. Allow the analyzer to cool before continuing.
- c. Open the vacuum manifold.
- d. Disconnect any gas lines or electrical connections to the analyzer assembly.
- e. Remove the analyzer assembly from the manifold.
- f. Disassemble the ion trap and separate the ring and center electrodes.
- g. Mix a slurry of aluminum oxide and methanol.
- h. Thoroughly clean the surfaces of the electrodes using the slurry. Using a cotton-tipped applicator, clean all dark or discolored areas. Warning: Only clean metal surfaces.
- i. Place the parts in a beaker with deionized water and sonicate for one minute.
- j. Thoroughly rinse the parts with deionized water followed by methanol.
- k. Reassemble the ion trap and analyzer assembly.
- l. Place the analyzer assembly in the manifold and reconnect any gas lines and electrical connections.

- m. Seal the manifold.
- n. Turn on the main power and pump down the system, observing for vacuum leaks.

8.2.3.2 Cleaning a Quadrupole

- a. Vent the MS system and turn off the main power.
- b. Allow the analyzer to cool before continuing.
- b. Open the vacuum manifold.
- d. Disconnect any gas lines or electrical connections to the quadrupole.
- e. Remove the quadrupole from the manifold.
- f. Mix a slurry of aluminum oxide and methanol.
- g. Thoroughly clean the first inch of the inner surfaces of the quadrupole with the slurry. Using a cotton-tipped applicator, clean area of interest, particularly dark or discolored areas. Warning: only clean inner metal surfaces.
- h. Rinse by running water through the inside of the quadrupole for several minutes, until all aluminum oxide has been removed.
- i. Thoroughly rinse with methanol and allow quadrupole to dry.
- j. Place the quadrupole in the manifold and reconnect any gas lines and electrical connections.
- k. Seal the manifold.
- l. Turn on the main power and pump down the system, observing for vacuum leaks.

8.2.4 Filament Replacement

Generally, a broken filament results in a total loss of ions rather than degraded system performance. To replace a filament:

- a. Vent the MS system and turn off the main power.
- b. Allow the source to cool before continuing.
- c. Open the vacuum manifold.

- d. Disconnect any gas lines or electrical connections to the source.
- e. Loosen and/or remove source retaining bolts and clips.
- f. Remove the source.
- g. Disassemble the source in order to expose the filament.
- h. Unplug the old filament and replace it with a new one.
- i. Re-assemble the source.
- j. Place the source in the manifold and secure.
- k. Reconnect all gas lines and electrical connections.
- l. Seal the manifold.
- m. Turn on the main power and pump down the system, observing for vacuum leaks.

9 Instrumental Conditions

Refer to the appropriate procedures outlined in section 7, manufacturer's instrument manuals, or the instrument's performance monitoring protocol for specific instrumental conditions to be used during maintenance procedures.

10 Decision Criteria

Every performance monitoring protocol will have specific decision criteria to determine if the instrument is operating properly. If these should fail, the operator should refer to the 'Decision Criteria' section of the specific performance monitoring protocol. Additional information is provided in the 'Corrective Maintenance' section of the "General Instrument Maintenance Protocol".

11 Calculations

Not applicable.

12 Measurement Uncertainty

Not applicable.

13 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of any instrument. Instrument-specific limitations will be specified in the appropriate SOP.

14 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

15 References

Instrument Operation and Systems Support SOP Manual.

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual.*

"Gas Chromatograph General Maintenance Protocol" (Inst 002) *Instrument Operation and Systems Support SOP Manual.*

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and Systems Support SOP Manual.*

FBI Laboratory Safety Manual.

FBI Laboratory Quality Assurance Manual.

FBI Laboratory Operations Manual.

Rev. #	Issue Date	History
0	06/21/06	New document which replaces original also titled "Mass Spectrometer General Maintenance Protocol."
1	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Changed 'Chemistry Unit' to 'Quantico laboratory' and added 'appropriate instrument support personnel' in Section 8.2.3. Updated abbreviation for IOSS in Section 15 and header. Updated section heading for Section 7 and updated wording in Section 10 for clarification

Approval

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Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Toxicology
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Metallurgy
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Paints and Polymers
Technical Leader:

Date: 09/28/2018

Fire Debris Technical
Leader:

Date: 09/28/2018

Explosives (Chemistry)
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Chemnet Instrument Data Archiving Protocol

1 Scope

The purpose of this document is to provide a uniform protocol for the storage of raw electronic data generated by instrumentation. Data files take up a great deal of the hard disk space and cause a variety of system problems when allowed to remain on system hard drives for an extended period of time. Therefore, a protocol has been established to purge old data and to provide optional methods for permanent data archiving. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, paint, explosives (chemistry), fire debris, and Chemistry Unit general physical and chemical analysis.

2 Principle

As stated in individual protocols, processed data files are printed (either hard copy or pdf) and kept with case files or instrument QA/QC logs, as required. Once this processed data has been printed and filed properly, the printed material is considered the "official" copy. However, where possible, raw data files are kept and archived. The majority of the instrument computers are connected to Chemnet, a closed, unclassified TCP/IP network.

This protocol outlines the amount of time that data may be stored on an instrument computer and optional methods of data archiving. The term 'data' used in this SOP may refer to 'raw data,' 'data file,' 'file,' directories, other instrument files including methods, sequences, layouts, and reports and may refer to the location of the analysis data on an instrument computer hard drive. This protocol only applies to instruments that are capable of storing data to a workstation hard drive.

3 Equipment/Materials/Reagents

- a. Chemnet storage servers and data jukeboxes
- b. CD-R, DVD-R, BD-R disks

4 Standards and Controls

Not applicable.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Hard Drive Storage

- a. Data will be kept on instrument computer hard drives for a period of approximately six months unless it is removed sooner by the originator of the data or it is archived to free-up memory on the hard drive. Where applicable, the performance monitoring protocols direct the operator to view and record the remaining available disk space daily and to contact appropriate instrument support personnel if insufficient space is available.
- b. Checks will be made as needed by appropriate instrument support personnel to remove data files dated older than six months on heavily used instrument workstations in order to maintain sufficient hard disk memory space.

6.2 Data Archiving

6.2.1 IOSS Manager, or Appropriate Instrument Support Personnel

- a. When the data stored on an instrument hard drive becomes excessive (typically indicated by the availability of less than 100 MB of free disk space), data files will be transferred to the Chemnet storage servers, by either manual or automated methods, for temporary storage.
- b. Periodically, the data located on the Chemnet servers will be transferred to CD-R, DVD-R, or BD-R disks for permanent storage/archival, by either manual or automated methods when necessary.
- c. Disks containing archived data are initially stored in the Chemnet jukeboxes for online access to the data. Periodically, to make room in the jukeboxes, older disks will be removed and stored in a locked cabinet.

6.2.2 Individual Instrument Operators

- a. If an individual operator, for any reason, wishes to archive data, the data should be removed from the hard drive and stored on another medium. This is only optional, and not a requirement.
- b. Methods of data archiving can be suggested by the IOSS Manager or appropriate instrument support personnel. The instrument operator can decide which type of media to use. However, CD-R, DVD-R, or BD-R disks are highly recommended.
- c. It is the responsibility of the individual instrument operator to store the archived data that they have generated.

7 Calculations

Not applicable.

8 Measurement Uncertainty

Not applicable.

9 Limitations

Not applicable.

10 Safety

Not applicable.

11 References

Instrument Operation and Systems Support SOP Manual.

Rev. #	Issue Date	History
0	06/21/06	New document which replaces original also titled "Chemistry Unit Data Archiving Policy."
1	10/04/18	Changed title to "Chemnet Data Archiving Protocol." Updated Section 1 Scope to include applicable disciplines/categories of testing. Added pdf printing in Section 2. Deleted Calibration section and renumbered. Updated Sections 3a. and 7.2.1a. to include storage servers and jukeboxes. Updated Sections 3b, 7.2.1b, and 7.2.2b. to include BD-R disks. Changed 'IOSS' to 'appropriate instrument support personnel' in Sections 7.1 a, 7.1 b, 7.2.1, and 7.2.2 b. Updated heading in Section 6. Updated abbreviation for IOSS in Section 12 and header.

Approval

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Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Toxicology
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Date: 09/28/2018

Metallurgy
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Date: 09/28/2018

Paints and Polymers
Technical Leader:

Date: 09/28/2018

Fire Debris Technical
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Date: 09/28/2018

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Date: 09/28/2018

Redacted - Signatures on File

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Thermo TSQ Quantum GC/MS (EI/CI)

1 Scope

This document addresses the performance monitoring (QA/QC) of the Thermo TSQ Quantum GC/MS (EI/CI) System. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, explosives (chemistry), fire debris, and Chemistry Unit general physical and chemical analysis.

2 Principle

The Thermo TSQ Quantum GC/MS consists of a TraceGC Gas Chromatograph (GC) and a Triple Stage Quadrupole (TSQ) Quantum Mass Spectrometer (MS). These two instruments work in tandem and are referred to as the TSQ Quantum. The instrument is configured with a combination electron impact (EI) ionization and chemical impact (CI) ionization source using an interchangeable ion volume system.

When the instrument is in EI mode, it is implied that an EI volume is being used and that the reagent gas is off. Alternatively, when in CI mode, it is implied that a CI volume is being used and the reagent gas is on. The instrument can also be used with a Solids Probe. Solids Probe is a sample introduction technique utilizing the mass spectrometer for analysis and can be in either EI or CI mode. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Thermo TSQ Quantum, Thermo TraceGC Ultra, and data system with XCalibur software (or equivalent)
- b. Autosampler - CTC A200S or "Pal" Series automated sampler, accessories, and software (or equivalent)
- c. GC Column - Agilent DB-5MS, 30 m, 0.25 mm i.d., 0.25 μ m film (or equivalent)
- d. Carrier Gas - Helium, 99.99% (high purity)
- e. CI Reagent Gas - Methane, 99.99% (high purity)
- f. Chloroform, GC grade
- g. Lidocaine HCl (Sigma or equivalent)

- h. Tributoxyethyl Phosphate (TBEP) (Chem Service or equivalent)
- i. Perfluorotributylamine (PFTBA, FC-43) (Agilent or equivalent)
- j. Analytical balance
- k. Volumetric flask
- l. Autosampler vials - 2 mL GC vials, crimp or screw top, with or without 100-500 μ L inserts (Agilent or equivalent)
- m. Injection port liners - 3 mm split-splitless, tapered, with or without glass wool (Restek or equivalent)
- n. Injection port septa - low-bleed 17 mm (Restek or equivalent)
- o. Autosampler syringes - SGE Analytical gas tight 10 μ L (or equivalent)

4 Standards and Controls

4.1 Testmix (0.05 mg/mL each of Lidocaine and TBEP)

The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. To prepare, weigh 5.8 mg Lidocaine HCl and 5 mg TBEP into a 100-mL volumetric flask. Bring to the mark with chloroform and mix well. Store the solution in the refrigerator. It has a shelf-life of three years. This preparation may be appropriately scaled up.

4.2 PFTBA Tuning Solution

The PFTBA tuning solution is used for tuning the mass spectrometer and verifying mass calibration. It is supplied by the instrument manufacturer and does not expire. It is stored in a glass container attached to the TSQ. Under normal conditions, this should not need to be refilled.

5 Calibration

Not applicable.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Check to ensure that the GC wash vials are filled, the waste vials are empty, and all are in the appropriate positions.
- b. Record the remaining disk space on the hard drive. Use Windows Explorer or XCalibur to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- c. Record the line pressure of the building helium supply (carrier gas). The regulator should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the helium is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.
- d. If using CI mode, record the tank pressure of the methane tank (reagent gas). Change the tank if less than 100 p.s.i. remaining.
- e. Check the Ion Gauge to ensure that there are no significant leaks in the system. Do not use if the pressure is higher than 1×10^{-4} torr with the reagent gas off.
- f. Prepare instrument for EI mode or CI mode. In the Quantum Tune Master software, select the ionization mode under 'Setup.' Open both the corresponding tune and calibration files (such as EI_TUNE or PICI_TUNE). Insert the correct ion volume. Check that the reagent gas is ON with a value of 2.0 for CI mode and OFF for EI mode.
- g. Perform an analysis of the testmix. Open the appropriate testmix instrument method (such as 'TestmixEI' or 'TestmixCI'), and verify the parameters as listed in the 'Instrumental Conditions' section of this protocol. Set up a sequence, load the autosampler with a vial containing the testmix, and start the analysis. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the following information for the testmix:
 - For EI: RIC of m/z 86, RIC of m/z 299, and TIC. Label the peaks with scan number and/or retention time.
 - For CI: RIC of m/z 235, RIC of m/z 399, and TIC. Label the peaks with scan number and/or retention time.
 - Complete mass spectrum of both Lidocaine and TBEP.

- h. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

7.2 As Needed Checks

The following steps will be performed as needed based on system performance. Indicate completion in the appropriate QA/QC log.

- a. Replace the septum in the GC injection port.
- b. Replace the liner within the GC injection port.
- c. Check the GC syringe in the autosampler. Replace if needed.
- d. Check the internal bungee cords in the autosampler. Replace if needed.
- e. Appropriate instrument support personnel or trained operator: Tune the mass spectrometer. Perform the following procedure for both EI and CI modes:
 1. Save the current tunes as backups with filenames such as 'EI_TUNE_BACKUP' or 'PICI_TUNE_BACKUP.'
 2. Perform a standard tune on both Q1 and Q3.
 3. Save the tune files when completed with filenames such as 'EI_TUNE' or 'PICI_TUNE'.
 4. Manual optimization of parameters may be performed to fine-tune the MS.
 5. Acquire a mass spectrum of the PFTBA for Q1 and Q3 in EI, Positive Ion CI, and Negative Ion CI ranging from 50 to 650 m/z:
 - For EI: Collect approximately 20 scans for each quadrupole under the filename "tunespec1".
 - For Positive Ion CI: Collect approximately 20 scans for each quadrupole under the filename "tunespec2".
 - For Negative Ion CI: Collect approximately 20 scans for each quadrupole under the filename "tunespec3".
 6. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the mass spectrum from each.
- f. If all requirements are within specification, prepare the documentation as outlined in

the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be taken.

8 Instrumental Conditions

8.1 Gas Chromatograph

Oven

Initial Temp: 60°C
Initial Time: 2.0 min
Ramp: 35°C/min
Final Temp: 250°C
Hold Time: 10.0 min

Inlet/Injector

Inj Vol: 1.0 µL
Mode: Splitless
Inlet Temp: 220°C

Column

Type: DB-5(MS)
Length: 30 m
Diameter: 0.25 mm
Film Thickness: 0.25 µm
Flow Mode: Constant Flow, Vacuum Compensation
Pressure: 1 mL/min
Carrier Gas: Helium

8.2 Mass Spectrometer

Solvent Delay: 5.0 min
Scan Mode: Full Scan
Scan Range: 50-500 m/z (EI)
100-500 m/z (CI)

Temperatures

Transfer Line: 280°C
Source: 185°C

9 Decision Criteria

9.1 Testmix

Verify the results of the testmix.

- a. In order for the instrument to be considered in good operating condition, both Lidocaine and TBEP should generate well-resolved, Gaussian-shaped peaks with baseline separation.
- b. A SNR of 3:1 will be the minimum response necessary to consider a response a peak.
- c. There should be no significant extraneous peaks in the chromatogram.
- d. The retention times of each component should be similar as compared to previous analyses (unless GC maintenance has been performed, such as column clipping or replacement).
- e. Check for the correct mass assignments for the mass spectra:
 - EI - Lidocaine ions 86 and 234 and TBEP ions 57, 199, and 299.
 - CI - Lidocaine ion 235 and TBEP ions 299 and 399.

9.2 Tune

Verify the results of the tune. Compare the results of the tune to previous tune results. The following are typical PFTBA values for the TSQ. If the observed PFTBA peaks are outside the values listed below, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be performed.

- a. PFTBA Tune: Mass assignments for m/z 69, 219, 414, 502, and 614
- b. Relative abundance:
 - EI: 69 or 219 base peak, 414 and 502 present
 - CI+: 414 base peak, 219, 69, and 614 present
 - CI-: 633 or 452 base peak, 414 present

10 Calculations

Not applicable.

11 Measurement Uncertainty

Not applicable.

12 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

14 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Gas Chromatograph General Maintenance Protocol" (Inst 002) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	06/21/06	New document which replaces original titled "Performance Monitoring Protocol (QA/QC) for the Finnigan TSQ GC/MS (EI/CI)."
1	05/01/08	Changed 'monthly' checks to 'as needed' in section 7.2. Corrected column information and instrument conditions in section 3 and 8.1. Updated ion ratios in section 9.2.
2	08/19/09	Updated with manufacturer's current name and model name (following replacement of outdated instrument with new model) in title and sections 1, 2, and 3a. Removed reference to old software in 7.1b. Updated steps for using new software in 7.1f. Added check of bungee cords in 7.2d. Removed reference to old software in 7.2e.iv. Changed final temp in 8.1 and transfer line temp in 8.2 to match other GC/MS instruments.
3	04/01/11	Updated manufacturer information on autosampler syringe in section 3. Changed PFTBA tuning decision ion for CI+ mode from m/z 619 to m/z 614 in section 9.2b.
4	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Updated heading in Section 6. Added 'appropriate instrument support personnel' to Sections 7.1 c & h, 7.2 e & f and 9.2. Updated Section 9.1 b & c to account for instrument variation and maintenance. Updated Section 14 and header to 'Instrument Operation and Systems Support.'

Approval

Redacted - Signatures on File


Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Toxicology
Technical Leader:

Date: 09/28/2018

Fire Debris Technical
Leader:

 Date: 09/28/2018

Explosives (Chemistry)
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Pyrolysis-GC/MS (Py-GC/MS)

1 Scope

This document addresses the performance monitoring (QA/QC) of a GC/MS (EI) with a pyrolysis autosampler. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Paint and Chemistry Unit general physical and chemical analysis.

2 Principle

The pyrolysis-GC/MS system consists of a Gas Chromatograph (GC) with a single quadrupole Mass Selective Detector (MSD) and a pyrolysis autosampler. The instrument is configured with a dedicated electron impact ionization (EI) source. It may also be referred to as a 'Py-MSD.' Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

Pyrolysis is a technique used to break chemical bonds using in macromolecules using thermal energy. The pyrolysis products, known as pyrolyzates, are introduced onto the GC column by the carrier gas through a heated transfer line. The elution order of the pyrolyzates by gas chromatography and the structural information ascertained from the observed fragmentation pattern of each can be used to identify the pyrolyzates present.

3 Equipment/Materials/Reagents

- a. Instrumentation - Gas Chromatograph, Mass Selective Detector with EI Source, and Software (Agilent or equivalent)
- b. Autosampler - Pyrolysis Autosampler, accessories, and software (Frontier, or equivalent)
- c. GC Column – mid-polarity capillary column (HP-5, 30 m, 0.25 mm i.d., 0.25 μ m film or equivalent)
- d. Carrier Gas - Helium, 99.99% (high purity)
- e. Sample holder - alloyed metal cups (Frontier or equivalent)
- f. Cleaning apparatus for sample holders (e.g., aluminum block, muffle furnace, small butane torch, sample cup inspector, sample cup holder)
- g. Polystyrene Pellets (Scientific Polymer Products, Inc. or equivalent)

- h. High Density Polyethylene Pellets (Scientific Polymer Products, Inc. or equivalent)
- i. Perfluorotributylamine (PFTBA, FC-43) (Agilent or equivalent)
- j. Stereo-microscope (~ 6 to ~ 50x) with appropriate lighting (annular ring light or fiber optic light)
- k. Scalpel with blades
- l. Wire probe
- m. Tweezers
- n. Glass microscope slides
- o. Analytical microbalance

4 Standards and Controls

4.1 Performance Verification Standards

4.1.1 Polystyrene Standard (Daily QA/QC Standard)

The polystyrene is used to assess daily operating performance, mass assignment, and continued integrity of the system. It can be purchased as a standard in pellet form. Prepare for analysis by cutting a polystyrene pellet to obtain a size that will provide an adequate signal. Place the polystyrene in the bottom of a sample cup.

4.1.2 Polyethylene Standard (Monthly QA/QC Standard)

The polyethylene is used to assess monthly operating performance, column selectivity, and continued integrity of the system. It can be purchased as a standard in pellet form. Prepare by cutting a polyethylene pellet to obtain a size that will provide an adequate signal. Place the polyethylene in the bottom of a sample cup.

4.2 PFTBA Tuning Solution

The PFTBA tuning solution is used for tuning the mass spectrometer and verifying mass calibration. It is supplied by the instrument manufacturer and does not expire. It is stored in a glass container attached to the MSD. Under normal conditions, this should not need to be refilled.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log.

- a. Perform a tune of the instrument. If Autotune (ATUNE) is selected, the mass spectrometer will tune itself using PFTBA. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the tune file (ATUNE) when completed.
- b. Perform an analysis of a blank followed by a polystyrene standard prior to sample analysis. Open the appropriate instrument method, and verify the parameters as listed in the 'Instrumental Conditions' section of this protocol. Set up a sequence, load the autosampler with a blank and a sample vessel containing the polystyrene, and start the analysis. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC and mass spectra for the polystyrene as well as for the prior blank.
- c. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.2 Monthly Checks

The following steps will be performed monthly. Enter the appropriate information in the QA/QC log to indicate completion.

- a. Perform an analysis of a blank followed by the polyethylene standard. Open the appropriate instrument method, and verify the parameters as listed in the 'Instrumental Conditions' section of this protocol. Set up a sequence, load the autosampler with a blank and a sample vessel containing the polyethylene, and start the analysis. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC for the polyethylene as well as for the prior blank.
- b. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective action to be taken.

7 Instrumental Conditions

Refer to the “Pyrolysis-Gas Chromatography/Mass Spectrometry Analysis of Paints, Tapes, and Polymers” SOP for recommended instrument conditions.

8 Decision Criteria

8.1 Tune

Verify the results of the tune. Compare the results of the tune to previous tune results. Significant voltage increases or changes in the isotope ratios indicate the need to initiate corrective maintenance procedures. The following are typical ATUNE values for the MSD:

- a. PFTBA Tune: $m/z \pm 0.4$ for m/z 69, 219, and 502
- b. Peak width: 0.45-0.65
- c. Relative abundance: 69 greater than 50%
219 greater than 50%
502 greater than 1%

8.2 Polystyrene and Polyethylene

Verify the results.

- a. In order for the instrument to be considered in good operating condition, all components should generate well-resolved, Gaussian-shaped peaks with baseline separation.
- b. A SNR of 3:1 will be the minimum response necessary to consider a response a peak.
- c. There should be no significant unrelated peaks in the pyrogram.
- d. Ideally, the blank preceding the performance verification standard should not exhibit any chromatographic peaks greater than the CO₂ response; if extraneous peaks are present but explainable (e.g., siloxanes), this should be noted on the blank printout for technical review.
- e. The retention times of each component should be similar as compared to previous analyses (unless GC maintenance has been performed, such as column clipping or replacement).
- f. For polystyrene, check for the correct mass assignments in the mass spectrum, and compare the fragmentation patterns with previous analyses.

- Monomer ions - 50, 51, 77, 78, 103, and 104 (base peak).
- Dimer ions - 65, 91 (base peak), 104, 115, 117, 130, 193, and 208.
- Trimer ions - 91 (base peak), 115, 117, 194, 207, and 312.

g. For polyethylene, observe that there is baseline separation between the groupings of dialkenes, alkenes, and alkanes. Furthermore, compare with previous analyses.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Not applicable.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher, and areas of the pyrolysis autosampler reach temperatures in excess of 800°C. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

13 References

Manufacturer(s)'s Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Gas Chromatograph General Maintenance Protocol" (Inst 002) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

“Pyrolysis-Gas Chromatography/Mass Spectrometry Analysis of Paints, Tapes, and Polymers”
(PPSU 201) *Paints and Polymers SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
4	03/26/15	Changed scope and principle to be non-instrument specific; edited equipment list in Section 3 to align with order in PPSU 201; edited Sections 4.1.1 and 4.1.2 to be inclusive of different sample intro methodologies for different instrument types; removed recording of disk space from Section 6.1 as well as save to disk instruction since program autosaves this info; changed reference for Decision Criteria in Section 6.2a to PPSU SOP 201. Removed sections 7.1-7.3 because the information is covered in PPSU SOP 201 for this technique; also changed "specific" to "recommended" in Section 7 since the parameters are a guide for use of two different instrument systems, no longer specific to one instrument type/brand. Minor grammatical editing throughout. Section 8.2c simplified language, 8.2d accounted for explainable extraneous peaks and 8.2e changed allowable deviation range for RT to align with other subunits. Updated references.
5	10/04/18	Updated Section 1 Scope to include disciplines/categories of testing. Removed all references to 'CDS' in Section 3. Updated sample placement in Sections 4.1.1 and 4.1.2. Updated to 'appropriate instrument support personnel' in Sections 6.1 c and 6.2 b. Updated Section 8.2 c & e to account for instrument variation and maintenance. Updated 'Instrument Operation and Systems Support' in Section 13 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
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Date: 09/28/2018

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Technical Leader:

Date: 09/28/2018

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QA Approval

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Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Agilent Headspace GC/MS

1 Scope

This document addresses the performance monitoring (QA/QC) of the Agilent GC/MS System with a Headspace Autosampler, which may include optional detectors, such as a Nitrogen Phosphorus Detector (NPD). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, explosives (chemistry), fire debris, and Chemistry Unit general physical and chemical analysis.

2 Principle

The Agilent Headspace GC/MS is a gas chromatograph (GC) with a headspace autosampler. The system may also be equipped with an additional detector, such as an NPD. A headspace autosampler is a device used to sample the gas phase volatile analytes within a sealed vial. This sampling is transferred to the inlet of the GC and onto a column where the components are separated and sent to the detector. There may be two columns in the Agilent GC (labeled front and back), each leading to respective detectors. The front column is a capillary column which leads to a single quadrupole Mass Selective Detector (MSD) Mass Spectrometer. The MSD is configured with a dedicated electron impact ionization (EI) source in the mass spectrometer, and is referred to as the HS-MSD throughout this document. The back column leads to an NPD. This portion of the instrument is referred to as the HS-NPD throughout this document. The headspace autosampler can be configured to inject into either inlet.

This performance monitoring protocol is based upon the manufacturer's recommendations. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Agilent 7890 Gas Chromatograph, Agilent 5975 or 5977 Mass Selective Detector with EI Source, Nitrogen-Phosphorus Detector (if equipped) and Chemstation Software, Gerstel Master, and/or Gerstel Maestro Software (or equivalent)
- b. Autosampler - Gerstel "MPS2" or CTC "Pal" Series automated sampler, accessories, and software (or equivalent)
- c. Capillary GC Columns:
Agilent DB-624, 30 m, 0.25 mm i.d., 1.4 µm film (or equivalent) (MSD)
Restek RT-QS-Bond 30 m, 0.32 mm i.d., 10 µm film (or equivalent) (NPD)

- d. Carrier Gases:
Helium, 99.99% (high purity) (MSD)
Nitrogen, 99.99% (high purity) (NPD)
- e. Perfluorotributylamine (PFTBA, FC-43) (Agilent or equivalent)
- f. Hydrogen gas (high purity)
- g. Compressed air
- h. Ethanol (200 Proof)
- i. Isopropanol (HPLC Grade)
- j. Chloroform (HPLC Grade)
- k. Methanol (HPLC Grade)
- l. Methyl Ethyl Ketone (HPLC Grade)
- m. Nitromethane (HPLC Grade)
- n. Acetone (HPLC Grade)
- o. Toluene (HPLC Grade)
- p. Deionized Water, 18 M Ω ·cm Milli-Q or equivalent
- q. Potassium cyanide (Reagent Grade)
- r. Sodium hydroxide (Reagent Grade)
- s. ~5 N (20% w/v) Sodium Hydroxide (NaOH):
To a 100-mL beaker or Erlenmeyer flask, add 60 mL water and 20 g sodium hydroxide. Mix well to dissolve and bring to volume with deionized water. Store in a Nalgene container at room temperature. Stable 1 year.
- t. Acetonitrile (HPLC Grade)
- u. 5 N Sulfuric Acid (H₂SO₄) (Reagent Grade)
- v. Autosampler vials - 10 or 20 mL crimp-top headspace autosampler vials or appropriate headspace vials for CTC "Pal" Series autosamplers (Gerstel or equivalent)

- w. Injection port septa - standard low-bleed 11 mm (Agilent or equivalent)
- x. Injection port liners - 4 mm split-splitless, tapered, with or without glass wool (Agilent or equivalent)
- y. Autosampler syringes - 2.5 mL headspace and 1 mL liquid syringes (Gerstel or equivalent)

4 Standards and Controls

The testmix is used to assess daily operating performance and continued integrity of the system. It will be analyzed and evaluated prior to the analysis of evidence.

4.1 Testmix (Toxicology/General Chemistry) for HS-MSD

Refer to the analyte-specific SOP for unique samples, such as GHB, for the preparation of the positive control standard which will be used as the testmix.

For general volatiles analysis, prepare the testmix by adding 500 mL of deionized water into a 1000-mL volumetric flask. Add 0.1 mL each of ethanol and isopropanol. Add 0.01 mL of chloroform. Bring to the mark with deionized water. Store refrigerated in glass or plastic. Stable for one year. Record preparation in the Reagent Log.

Transfer 0.5 mL of the solution into a 10-mL headspace vial. Alternatively, transfer 1.0 mL of the solution into a 20-mL headspace vial.

4.2 Testmix (Explosives Chemistry) for HS-MSD

Prepare the testmix by adding 50 mL of deionized water into a 100-mL volumetric flask. Add 0.01 mL each of methanol, ethanol, isopropanol, methyl ethyl ketone, nitromethane, acetone, and toluene. Bring to the mark with deionized water. Store refrigerated in a tightly sealed container. Stable for two years. Record preparation in the Reagent Log. This preparation may be appropriately scaled.

Refer to the analyte-specific SOP for unique samples, such as TATP, for the preparation of the positive control standard which will be used as the testmix.

4.3 Testmix (Cyanide Headspace Testmix) for HS-NPD

- a. Cyanide Stock Standard (0.2 mg/mL):
Prepared by adding 50 mg of potassium cyanide to a 100-mL volumetric flask containing 2 mL of ~5 N NaOH. Dilute to volume with deionized water and mix thoroughly. Store at room temperature in a tightly sealed glass or plastic container. Stable for 6 months.

- b. 0.04% Acetonitrile (v/v) (Internal Standard):
Add 40 µL acetonitrile to about 90 mL deionized water in a 100-mL volumetric flask. Dilute to volume with deionized water and mix thoroughly. Store at room temperature in a tightly sealed glass or plastic container. Stable at least one year.
- c. Aqueous Positive Control (10 µg/mL cyanide):
Prepared by adding 5 mL of the Cyanide Stock Standard into to a 100-mL volumetric flask. Dilute to volume with deionized water and mix thoroughly. Prepare fresh.
- d. Performance Verification Sample:
Measure 0.5 mL of the Aqueous Positive Control and 50 µL of 0.04% acetonitrile (Internal Standard) into a 20-mL headspace vial and cap. Using a 2.5 cc syringe, inject 0.5 mL of 5 N H₂SO₄ into the vial and thoroughly vortex the sample to uniformly distribute the acid. Wipe any residual H₂SO₄ from the septum and/or cap. Allow the sample to equilibrate at room temperature for 30 minutes.

4.4 PFTBA Tuning Solution for HS-MSD

The PFTBA tuning solution is used for tuning the mass spectrometer and verifying mass calibration. It is supplied by the instrument manufacturer and does not expire. It is stored in a glass container attached to the MSD. Under normal conditions, this should not need to be refilled.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks – HS-MSD

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- b. Record the line pressure of the building helium supply (carrier gas). The regulator should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the helium is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.
- c. Check the Ion Gauge to ensure that there are no significant leaks in the system. Do

not use if the pressure is higher than 6×10^{-5} torr.

- d. Perform a tune of the instrument. If Autotune (ATUNE) is selected, the mass spectrometer will tune itself using PFTBA. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, save and print the tune file (ATUNE) when completed.
- e. Refer to the analyte-specific SOP for unique samples, such as GHB or TATP, for the appropriate procedure, instrumental conditions, and decision criteria for performing an analysis of the testmix. For general volatiles analysis (Toxicology, General Chemistry, or Explosives Chemistry), perform an analysis of the headspace above the testmix prior to the analysis of evidence. Open the appropriate testmix instrument method, and verify the parameters as listed in the 'Instrumental Conditions' section of this protocol. Set up a sequence, load the autosampler with a vial containing the appropriate testmix, and start the analysis. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC and representative mass spectra.
- f. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.2 Daily Checks – HS-NPD

The following steps will be performed daily. Enter the appropriate information in the QA/QC log.

- a. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- b. Record the line pressure of the building nitrogen supply (carrier gas). The regulator should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the instrument is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. is remaining.
- c. Ensure that the autosampler injects into the appropriate GC inlet.
- d. Ensure that the NPD is operational using the front panel controls of the GC.
- e. Prepare a Performance Verification Sample as directed in Section 4.3.d and analyze as directed in Section 8.2.
- f. Evaluate the results using the 'Decision Criteria' section of this SOP. If the results are acceptable, print the chromatogram(s) for the performance verification standard.

- g. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.3 As Needed Checks

The following steps will be performed as needed based on system performance. Enter the appropriate information in the QA/QC log to indicate completion.

- a. Replace the septum in the GC injection port.
- b. Replace the liner within the GC injection port.
- c. Check the GC syringe in the autosampler. Replace if needed.
- d. Check the internal bungee cords in the autosampler (if equipped). Replace if needed.
- e. Check the plungers in each autosampler syringe. Replace if needed.

7 Instrumental Conditions

7.1 HS-MSD (Toxicology/General Chemistry) Testmix Parameters

7.1.1 Headspace Sampler Parameters

Incubation temperature:	80°C
Incubation time:	10 min
Agitator speed:	300 RPM
Agitation timing:	10 sec on 1 sec off
Syringe temperature:	90°C
Sample fill volume:	1.0 mL
Sample fill rate:	1.0 mL/sec
Sample fill strokes:	5
Sample injection speed:	1.0 mL/sec
Syringe flush time:	1.0 min

7.1.2 Gas Chromatograph Parameters

Oven

Temperature:	50°C for 3 min
Ramp:	10°C/min to 250°C for 5 min
Run time:	28 min
Equilibration time:	0 min

Column

Type: DB-624
Length: 30 m
Internal diameter: 0.25 mm
Film thickness: 1.4 µm

Inlet/Carrier

Inlet temperature: 150°C
Injection mode: Split
Carrier gas: Helium, 99.99% (split)
Carrier mode: Constant flow
Pressure: 6.5 psi
Split ratio: 10:1

7.1.3 Mass Spectrometer Parameters

Ionization mode: Electron impact
Scan mode: Full scan
Scan range: 27 – 400 m/z
Relative voltage: 106 V
Source temperature: 230°C
Transfer line temperature: 260°C
Quadrupole temperature: 150°C
Solvent delay: 1.75 min

7.2 HS-MSD (Explosives Chemistry) Testmix Parameters

7.2.1 Headspace Sampler Parameters

Incubation temperature: 80°C
Incubation time: 5 min
Agitator speed: 300 RPM
Agitation timing: 10 sec on
1 sec off
Syringe temperature: 90°C
Sample fill volume: 1.0 mL
Sample fill rate: 1.0 mL/sec
Sample fill strokes: 5
Sample injection speed: 1.0 mL/sec
Syringe flush time: 4.0 min

7.2.2 Gas Chromatograph Parameters

Oven

Temperature: 40°C for 4 min
Ramp: 10°C/min to 120°C for 0 min

Run time: 12 min
Equilibration time: 0.25 min

Column

Type: DB-624
Length: 30 m
Internal diameter: 0.25 mm
Film thickness: 1.4 μ m

Inlet/Carrier

Inlet temperature: 150°C
Injection mode: Split
Carrier gas: Helium, 99.99% (split)
Carrier mode: Constant pressure
Pressure: 5.3 psi
Split ratio: 10:1

7.2.3 Mass Spectrometer Parameters

Ionization mode: Electron impact
Scan mode: Full scan
Scan range: 29 – 400 m/z
Relative voltage: 106 V
Source temperature: 230°C
Transfer line temperature: 260°C
Quadrupole temperature: 150°C
Solvent delay: 2.0 min

7.3 HS-NPD Testmix Parameters

7.3.1 Headspace Sampler Parameters (NPD)

Syringe: 2.5 mL-HS
Oven / syringe temp.: 45°C / 55°C
Flush time: 4.0 min
Incubation time: 5.0 min
Agitator speed: 250 rpm
Agitation timing: 10 sec on
1 sec off
Injection volume: 250 μ L
Fill speed / strokes: 500 μ L/sec / 5
Incubation time: 5.0 min
Injection speed: 1000 μ L/sec
Injection penetration: 40 mm

7.3.2 Gas Chromatograph Parameters (NPD)

Oven

Temperature: 110°C for 0 min
Ramp: 4°C/min to 130°C for 5 min
Run time: 10 min
Equilibration time: 0.2 min

Column

Type: RT-QS-Bond
Length: 30 m
Internal diameter: 0.32 mm
Film thickness: 10 µm

Inlet and Carrier

Inlet temperature: 150°C
Injection mode: Purged
Carrier gas: Nitrogen
Carrier mode: Constant flow

7.3.3 Detector Parameters (NPD)

NPD

Temp.: 225°C
Offset: 20
Equilibration: 0.01 min
Air flow: 60 mL/min
Hydrogen flow: 3.0 mL/min
Electrometer: ON

8 Decision Criteria

8.1 HS-MSD Testmix

Verify the results of the testmix.

- a. In order for the instrument to be considered in good operating condition, all testmix components should generate well-resolved, Gaussian-shaped peaks with baseline separation.
- b. A SNR of 3:1 will be the minimum response necessary to consider a response a peak.
- c. There should be no significant extraneous peaks in the chromatogram. |
- d. The retention times of each component should be similar as compared to previous |

analyses (unless GC maintenance has been performed, such as column clipping or replacement).

- e. Check for the correct mass assignments for the mass spectra. In order for the MS to be considered in good operating condition, the correct mass assignments for each of the analytes in the appropriate testmix should be present. The following ions at m/z should be present:

Toxicology/General Chemistry

- ethanol (31, 45, 29)
- isopropanol (45, 43, 29)
- chloroform (47, 83, 85)

Explosives Chemistry

- methanol (31, 32, 29)
- ethanol (31, 45, 29)
- isopropanol (45, 43, 29)
- methyl ethyl ketone (43, 72, 29)
- nitromethane (30, 61, 46)
- acetone (43, 58)
- toluene (91, 92, 65)

8.2 MSD Tune

Verify the results of the tune. Compare the results of the tune to previous tune results. Significant voltage increases or changes in the isotope ratios indicate the need to initiate corrective maintenance procedures. The following are typical ATUNE values for the MSD:

- | | | |
|----|---------------------|--|
| a. | PFTBA Tune: | Mass ± 0.4 for m/z 69, 219, and 502 |
| b. | Peak width: | 0.45-0.65 |
| c. | Relative abundance: | 69 greater than 50%
219 greater than 50%
502 greater than 1% |

8.3 HS-NPD Testmix

The peaks of both cyanide and acetonitrile should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. Peak areas should compare favorably to previous analyses of the performance standard.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. Acid liberates hydrogen cyanide gas and care must be taken to isolate acid solutions from cyanide sources. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

13 References

Manufacturer(s)'s Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Gas Chromatograph General Maintenance Protocol" (Inst 002) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

"Volatile Chemicals by Automated Headspace GC/MS (EI)" (Tox 300) *Toxicology SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
6	04/29/16	Section 4.2 changed to reflect storage of testmix in refrigerator, and added statement to allow preparation to be appropriately scaled. Sections 4.2 and 6.1 additions to include analysis of TATP by following analyte specific SOP. Section 8, removed mass 15 from requirement for acetone.
7	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Updated Section 3 s. Changed to 'appropriate instrument support personnel' in Sections 6.1 b & f and 6.2 b & g. Updated vacuum to '6 x 10 ⁻⁵ torr' in 6.1 c. Updated Section 8.1 c & d to account for instrument variation and maintenance. Heading updated in Section 5. Updated 'Instrument Operation and Systems Support' in Section 13 and header.

Approval

Redacted - Signatures on File


Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Toxicology
Technical Leader:

Date: 09/28/2018

Fire Debris Technical
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 Date: 09/28/2018

Explosives (Chemistry)
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Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Agilent 7890/5975 Headspace GC/FID/MS

1 Scope

This document addresses the performance monitoring (QA/QC) of the Agilent 7890/5975 GC/FID/MS System with a headspace autosampler. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Toxicology and Chemistry Unit general physical and chemical analysis.

2 Principle

The Agilent 7890/5975 Headspace GC/FID/MS is a gas chromatograph (GC) with a headspace autosampler, two injectors, two columns, and two internal detectors. The headspace autosampler is a device used to sample the gas phase volatile analytes within a sealed vial. This sampling is transferred to the inlet of the GC and onto a column where the components are separated and sent to the detector. There are two columns in the Agilent 7890 GC (labeled front and back), each leading to respective detectors. The front is a capillary column and leads to a mass selective detector (MSD). The back is a capillary column which leads to a flame ionization detector (FID). The headspace autosampler can be configured to inject into either inlet.

This performance monitoring protocol is based upon the manufacturer's recommendations. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Agilent 7890 Gas Chromatograph, Flame Ionization Detector, Agilent 5975 Mass Selective Detector with EI Source, and Chemstation Software (or equivalent)
- b. Autosampler - Gerstel "MPS2" or CTC "Pal" Series automated sampler, accessories, and Gerstel Master/Gerstel Maestro Software (or equivalent)
- c. Capillary GC Columns:
Restek RTX BAC-2, 30 m, 0.32 mm i.d., 1.2 µm film (or equivalent) (FID)
Restek RTX BAC-1, 30 m, 0.32 mm i.d., 1.8 µm film (or equivalent) (MSD)
- d. Helium, 99.99% (high purity)
- e. Perfluorotributylamine (PFTBA, FC-43) (Agilent or equivalent)
- f. Hydrogen gas (high purity)

- g. Compressed air
- h. Methanol (Reagent Grade)
- i. Ethanol (200 Proof)
- j. Isopropanol (HPLC Grade)
- k. Acetone (HPLC Grade)
- l. Deionized Water, 18 MΩ·cm Milli-Q or equivalent
- m. 0.005% t-Butanol Internal Standard Solution (Reagent grade)
- n. Autosampler vials - 10 or 20 mL crimp-top headspace autosampler vials and caps for Gerstel MPS2 autosamplers (Gerstel or equivalent)
- o. Injection port septa - low-bleed 11 mm (Agilent or equivalent)
- p. Injection port liners - 1 mm split-splitless, (Restek or equivalent)
- q. Autosampler syringes - 2.5 mL headspace and 1 mL liquid syringes (Gerstel or equivalent)

4 Standards and Controls

4.1 Testmix (Volatiles Headspace Testmix)

The Volatiles Testmix is used to assess daily operating performance and continued integrity of the system. It will be analyzed and evaluated prior to the analysis of evidence.

- a. Volatiles Testmix Solution (0.1% v/v):
Add 25 mL deionized water to a 50 mL volumetric flask. Add 0.05 mL each methanol, ethanol, isopropanol and acetone. Bring to the mark with deionized water and mix well. Transfer 0.1 mL of the testmix solution and 1 mL of the t-Butanol Internal Standard Stock Solution (0.005%) to a 10 mL headspace vial and crimp. Repeat with the remaining testmix solution, for a total of nearly 200 testmix vials. Store vials refrigerated. Stable for at least one year.
- b. t-Butanol Internal Standard Solution (0.005% (w/v)):
Refer to Toxicology SOP "Ethanol and Common Volatiles in Biological Fluids by Automated Headspace GC/FID and GC/MS(EI)" (Tox 200) for preparation.

4.2 PFTBA Tuning Solution (MSD)

The PFTBA tuning solution is used for tuning the mass spectrometer and verifying mass

calibration. It is supplied by the instrument manufacturer and does not expire. It is stored in a glass container attached to the MSD. Under normal conditions, this should not need to be refilled.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log.

- a. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- b. Record the line pressure of the building helium supply (carrier gas). The regulator should read 70 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the instrument is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. is remaining.
- c. Ensure that the autosampler injects into the appropriate GC inlet.
- d. If using the FID, ensure that the FID flame is lit.
- e. If using the MSD, perform a tune of the instrument. If Autotune (ATUNE) is selected, the mass spectrometer will tune itself using PFTBA. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, save and print the tune file (ATUNE) when completed.
- f. Analyze the Volatiles Testmix and evaluate prior to the analysis of evidence.
- g. Evaluate the results using the 'Decision Criteria' section of this SOP. If the results are acceptable, print the chromatogram(s) and associated mass spectra (if applicable).
- h. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.2 As Needed Checks

The following steps are to be performed as needed. Record the appropriate information on the QA/QC log.

- a. Replace the septum in the GC injection port.
- b. Replace the liner within the GC injection port.
- c. Check the GC syringe in the autosampler. Replace if needed.
- d. Check the bungee cords in the autosampler (if equipped). Replace if needed.
- e. Check the plungers in each autosampler syringe. Replace if needed.

7 Instrumental Conditions

7.1 Headspace Sampler Parameters (FID)

Incubation temp.:	60°C
Incubation time:	30 min
Agitator speed:	250 rpm
Agitation timing:	10 sec on 1 sec off
Syringe:	2.5 mL-HS
Syringe temp.:	70°C
Sample fill volume:	500 µL
Sample fill rate:	500 µL/sec
Sample fill strokes:	5
Sample injection rate:	500 µL/sec
Syringe flush time:	2.0 min

7.2 Gas Chromatograph Parameters (FID)

Oven

Temp.:	40°C
Isothermal run time:	6 min
Equilibration time:	0.2 min

Column

Type:	RTX BAC-2
Length:	30 m
Internal diameter:	0.32 mm
Film thickness:	1.2 µm

Inlet and Carrier

Inlet temp.: 200°C
Injection mode: Split
Carrier gas: Helium
Carrier mode: Constant pressure
Pressure: 10.2 psi
Split ratio: 1:1

7.3 Headspace Sampler Parameters (MSD)

Incubation temp.: 60°C
Incubation time: 15 min
Agitator speed: 300 rpm
Agitation timing: 10 sec on
1 sec off
Syringe: 2.5 mL-HS
Syringe temp.: 70°C
Sample fill volume: 500 µL
Sample fill rate: 500 µL/sec
Sample fill strokes: 5
Sample injection rate: 500 µL/sec
Syringe flush time: 4.0 min

7.4 Gas Chromatograph Parameters (MSD)

Oven

Temp: 40°C
Isothermal run time: 6 min

Column

Type: RTX BAC-1
Length: 30 m
Internal diameter: 0.32 mm
Film thickness: 1.8 µm

Inlet and Carrier

Inlet temperature: 200°C
Injection mode: Split
Carrier gas: Helium
Carrier mode: Constant flow
Carrier flow: 1.23 mL/min
Split ratio: 10:1

7.5 Mass Spectrometer Parameters

Ionization mode:	Electron impact
Scan mode:	Full scan
Scan range:	27 – 100 m/z
Gain Factor:	1
Source temperature:	230°C
Transfer line temperature:	250°C
Quadrupole temperature:	150°C
Solvent delay:	1.3 min

8 Decision Criteria

8.1 Volatiles Testmix (FID)

The peaks of all four analytes should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. Peak areas should compare favorably to previous analyses of the performance standard.

8.2 Volatiles Testmix (MSD)

The peaks of all four analytes should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. Peak areas and mass spectra should compare favorably to previous analyses of the performance standard. At least one mass spectrum for a volatile in the testmix should be printed.

8.3 MSD Tune

Verify the results of the tune. Compare the results of the tune to previous tune results. Significant voltage increases or changes in the isotope ratios indicate the need to initiate corrective maintenance procedures. The following are typical ATUNE values for the MSD:

- a. PFTBA Tune: Mass ± 0.4 for m/z 69, 219, and 502
- b. Peak width: 0.45-0.65
- c. Relative abundance: 69 greater than 50%
219 greater than 50%
502 greater than 1%

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Not applicable.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

134 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Gas Chromatograph General Maintenance Protocol" (Inst 002) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

"Ethanol and Common Volatiles in Biological Fluids by Headspace GC/FID and GC/MS(EI)" (Tox 200) *Toxicology SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
1	10/13/11	Updated column details in Section 3c, deleted nitrogen from Section 3d, changed internal standard from acetonitrile to t-butanol internal in Section 3m, replaced CTC “Pal” Series vials with Gerstel vials in Section 3.n, and changed 4mm liners to 1mm liners in Section 3p. Updated numbering of Section 4. Updated Section 4.1a to use t-butanol internal standard. Added reference to Tox SOP in Sections 4.1b and 14. Added new Section 4.2 regarding PFTBA tuning solution. Changed carrier gas from nitrogen to helium in Section 7.1b. Added new Section 7.1e and renumbered subsequent sections. Removed references to internal standard solution in Sections 7.1f and 9.1. Deleted cycle time and updated fill volume, strokes, and injection rate in Section 8.1; run time, column details, inlet temp, carrier gas, and split ratio in Section 8.2; oven temp, run time, column details, inlet temp, and flow rate in Section 8.4; and transfer line temp and solvent delay in Section 8.5. Reformatted Section 8.3 to match 8.1 layout.
2	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Deleted Calibration section and renumbered. Updated heading in Section 5. Changed to ‘appropriate instrument support personnel’ in Section 7.1 b & h. Updated ‘Instrument Operation and Systems Support’ in Section 14 and header.

Approval

Toxicology Technical
Leader: _____

Redacted - Signatures on File

Date: 09/28/2018

Drug Chemistry/
General Chemistry
Technical Leader: _____

Date: 09/28/2018

IOSS Manager: _____

Date: 09/28/2018

Chemistry Unit Chief: _____

Date: 09/28/2018

QA Approval

Quality Manager: _____

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Agilent GC/MS

1 Scope

This document addresses the performance monitoring (QA/QC) of the Agilent GC/MS system which may include optional detectors, such as a Flame Ionization Detector (FID). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, paint, explosives (chemistry), fire debris, and Chemistry Unit general physical and chemical analysis.

2 Principle

The Agilent GC/MS system consists of an Agilent Gas Chromatograph (GC) with a single quadrupole Mass Selective Detector (MSD) Mass Spectrometer (MS). The system may also be equipped with an additional detector, such as an FID. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

The mass spectrometer will be configured to perform specific modes of ionization depending on which of the two types of ion sources is installed. If the electron impact (EI) ionization source is installed, only positive ion EI ionization analysis will be performed. However, if the chemical impact (CI) ionization source is installed, then either positive ion CI (PICI) or negative ion CI (NICI) analyses may be performed.

3 Equipment/Materials/Reagents

- a. Instrumentation - Agilent 7890 GC, 5975 or 5977 MSD with EI or CI Source, FID (if equipped), and MSD Chemstation software (or equivalent)
- b. Autosampler - Agilent ALS, CTC "Pal" Series, or Gerstel MPS automated sampler, accessories, and software (or equivalent)
- c. GC Column (MSD) – Agilent J&W DB-5 MS, 30 m, 0.25 mm i.d., 0.25 µm film (or equivalent)
- d. GC Column (FID) – Agilent J&W DB-5, 15 m, 0.25 mm i.d., 0.25 µm film (or equivalent)
- e. Carrier Gas - Helium, 99.99% (high purity)
- f. CI Reagent Gas - Methane, 99.99% (high purity)
- g. Compressed air

- h. Hydrogen Gas, 99.99% (high purity)
- i. Nitrogen Gas, 99.99% (high purity)
- j. Chloroform, GC grade
- k. Lidocaine HCl (Sigma or equivalent)
- l. Tributyoxyethyl Phosphate (TBEP) (Chem Service or equivalent)
- m. Perfluorotributylamine (PFTBA, FC-43) (Agilent or equivalent)
- n. Perfluoro-5,8-dimethyl-3,6,9-trioxidodecane (PFDTD) Tuning Solution (Agilent or equivalent)
- o. Analytical balance
- p. Volumetric flask
- q. Autosampler vials - 2 mL GC vials, crimp or screw top, with or without 100-500 μ L inserts (Agilent or equivalent)
- r. Injection port liners - 4 mm split-splitless, tapered, with or without glass wool (Agilent or equivalent)
- s. Injection port septa - standard low-bleed 11 mm (Agilent or equivalent)
- t. Autosampler syringes - Hamilton 701ASN 10 μ L (or equivalent)

4 Standards and Controls

4.1 PFTBA Tuning Solution

The PFTBA tuning solution is used for tuning the mass spectrometer and verifying mass assignment and accuracy when the EI source is installed. It is supplied by the instrument manufacturer and does not expire. It is stored in a glass container attached to the MSD. Under normal conditions, this should not need to be refilled.

4.2 PFDTD Tuning Solution

The PFDTD tuning solution is used for tuning the mass spectrometer and verifying mass assignment and accuracy when the CI source is installed. It is supplied by the instrument manufacturer and does not expire. It is stored in a glass container attached to the MSD. Under normal conditions, this should not need to be refilled.

4.3 Testmix (0.05 mg/mL each of Lidocaine and TBEP)

The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. To prepare, weigh 5.8 mg Lidocaine HCl and 5 mg TBEP into a 100-mL volumetric flask. Bring to the mark with chloroform and mix well. Store the solution in the refrigerator. It has a shelf-life of three years. This preparation may be appropriately scaled up.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks

The following steps will be performed daily, regardless of the ion source installed, mode of ionization, or the detector to be used. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Check to ensure that the GC wash vials are filled, the waste vials are empty, and all are in the appropriate positions.
- b. Record the remaining disk space on the hard drive. Use the Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- c. Record the line pressure of the building helium supply (carrier gas). The regulator should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the helium is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.

6.1.1 EI Source Daily Checks

If using the MSD with the EI source installed, perform the following steps:

- a. Check the Ion Gauge to ensure that there are no significant leaks in the system. Do not use if the source pressure is higher than 6×10^{-5} torr.
- b. Perform a tune of the instrument. If Autotune (ATUNE) is selected, the mass spectrometer will tune itself using PFTBA. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, save and print the tune file (ATUNE) when completed.

- c. Perform an analysis of the testmix. Open the appropriate testmix instrument method, and verify the parameters as listed in the 'Instrumental Conditions' section of this protocol. Set up a sequence, load the autosampler with a vial containing the testmix, and start the analysis. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC and mass spectra for both TBEP and Lidocaine.
- d. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.1.2 CI Source Daily Checks

If using the MSD with the CI source installed, perform the following steps:

- a. Record the tank pressure of the methane tank (reagent gas). Change the tank if less than 100 p.s.i. remaining.
- b. Check the Ion Gauge to ensure that there are no significant leaks in the system. Do not use if the source pressure is higher than the following:
 - PICI analysis - 6×10^{-4} torr with reagent gas on at approximately 20%
 - NICI analysis – 6×10^{-4} torr with reagent gas on at approximately 40%
- c. Perform an analysis of the testmix. Open the appropriate testmix instrument method, and verify the parameters as listed in the 'Instrumental Conditions' section of this protocol. Set up a sequence, load the autosampler with a vial containing the testmix, and start the analysis. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC and mass spectra for both TBEP and Lidocaine.
- d. If sample analyses will be performed using negative ion mode, no additional daily checks will be required.
- e. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.1.3 FID Daily Checks

If using the FID, perform the following steps:

- a. Record the line pressure of the hydrogen supply from the generator. The value should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel.

- b. Ensure that the FID flame is lit and functioning properly.
- c. Perform an analysis of the testmix. Open the appropriate testmix instrument method, and verify the parameters as listed in the 'Instrumental Conditions' section of this protocol. Set up a sequence, load the autosampler with a vial containing the testmix, and start the analysis. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the chromatogram.
- d. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.2 As Needed Checks

The following steps are to be performed as needed based on system performance. Indicate completion in the appropriate QA/QC log.

- a. Replace the septum in the GC injection port.
- b. Replace the liner within the GC injection port.
- c. Check the GC syringe in the autosampler. Replace if needed.
- d. Replace the autosampler bands (if equipped).
- e. Perform positive and/or negative CI autotune.

7 Instrumental Conditions

7.1 Gas Chromatograph/Mass Spectrometer

7.1.1 Gas Chromatograph

Oven

Initial Temp:	60°C
Initial Time:	2.0 min
Ramp:	35°C/min
Final Temp:	250°C
Hold Time:	10.0 min
Equilibration Time:	0.5 min

Inlet/Injector

Inj Vol: 1.0 µL
Mode: Splitless
Inlet Temp: 220°C

Column

Type: DB-5 MS
Length: 30 m
Diameter: 0.25 mm
Film Thickness: 0.25 µm
Mode: Constant Flow
Init Flow: 1.2 mL/min
Average Lin Velocity: 40 cm/sec
Carrier Gas: Helium

7.1.2 Mass Spectrometer

Solvent Delay: 3.0 min
Scan Mode: Full Scan
Scan Range: 50-500 m/z

Temperatures

Same for EI, PICI, NICI

Transfer Line: 280°C
Source: 200°C – 230 °C
Quad: 150°C

7.2 Gas Chromatograph/Flame Ionization Detector

7.2.1 Gas Chromatograph

Oven

Initial Temp: 100°C
Initial Time: 1.0 min
Ramp: 30°C/min
Final Temp: 260°C
Hold Time: 4.0 min
Equilibration Time: 0.5 min

Inlet/Injector

Inj Vol: 1.0 µL
Mode: Split
Inlet Temp: 220°C
Split Ratio: 50:1

Column

Type: DB-5
Length: 15 m
Diameter: 0.25 mm
Film Thickness: 0.25 μ m
Mode: Constant Flow
Init Flow: 1.0 mL/min
Carrier Gas: Helium

7.2.2 FID

Temperature: 280°C
Mode: Constant makeup flow
Hydrogen flow: 40.0 mL/min
Air flow: 450.0 mL/min
Makeup flow: 30.0 mL/min
Makeup gas: Nitrogen

8 Decision Criteria

8.1 Autotune

If an autotune of the mass spectrometer has been performed, verify the results below. Compare the results of the autotune to previous autotune results. Significant voltage increases or changes in the isotope ratios indicate the need to initiate corrective maintenance procedures.

8.1.1 Electron Impact Ion Mode

The following are typical electron impact ion autotune values for the MSD:

- a. PFTBA Tune: Mass \pm 0.4 for m/z 69, 219, and 502
- b. Peak width: 0.45-0.65
- c. Relative abundance: 69 greater than 50%
219 greater than 50%
502 greater than 1%

8.1.2 Positive Ion Chemical Ionization Mode

The following are typical positive ion autotune (PCICH₄) values for the MSD:

- a. PFDTD Tune: Mass \pm 0.4 for m/z 41, 267, 599
- b. Peak width: 0.45-0.65

- c. Relative abundance: 69 present
267 present
599 present

8.1.3 Negative Ion Chemical Ionization Mode

The following are typical negative ion autotune (NCICH₄) values for the MSD:

- a. PFDTD Tune: Mass \pm 0.4 for m/z 185, 283, 351
- b. Peak width: 0.45-0.65
- c. Relative abundance: 185 present
283 present
351 present

8.2 Testmix

8.2.1 Gas Chromatograph (regardless of detector type)

Verify the results of the testmix.

- a. In order for the instrument to be considered in good operating condition, both Lidocaine and TBEP should generate well-resolved, symmetrical peaks with baseline separation.
- b. A SNR of 3:1 will be the minimum response necessary to consider a response a peak.
- c. There should be no significant extraneous peaks in the chromatogram.
- d. The retention times of each component should be similar as compared to previous analyses (unless GC maintenance has been performed, such as column clipping or replacement).

8.2.2 Mass Spectrometer

In addition to the criteria in section 9.2.1, check the following criteria when using the mass spectrometer:

- a. Check for the correct mass assignments for the mass spectra, for EI:
- Lidocaine ions 86 and 234
 - TBEP ions 57, 199, and 299
- b. Check for the correct mass assignments for the mass spectra, for PICI Source:

- Lidocaine ion 235
- TBEP ions 199, 299, 399

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Gas Chromatograph General Maintenance Protocol" (Inst 002) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	10/01/12	New document titled "Performance Monitoring Protocol (QA/QC) for the Agilent GC/MS." This document replaces Inst 110 and Inst 306.
1	04/25/16	Section 3 updated for new model information. Section 7.1.2 changed to a range for the source temperature.
2	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Updated heading in Section 5. Added 'appropriate instrument support personnel' in Sections 6.1 c, 6.1.1 d, 6.1.2 e, and 6.1.3 a & d. Updated 8.2.1 c & d to account for instrument variation and maintenance. Updated to 'Instrument Operation and Systems Support' in Section 13 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018


Toxicology
Technical Leader:

Date: 09/28/2018

Paints and Polymers
Technical Leader:

Date: 09/28/2018

Fire Debris Technical
Leader:

 Date: 09/28/2018

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Date: 09/28/2018

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Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Agilent GC/MS Prior to Analysis of Toxicological Samples

1 Scope

This document addresses the performance monitoring (QA/QC) of the Agilent GC/MS system prior to the analysis of toxicological samples. The system may include optional detectors, such as a Thermal Conductivity Detector (TCD). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Toxicology.

2 Principle

The Agilent GC/MS (EI/CI) system consists of an Agilent Gas Chromatograph (GC) with a single quadrupole Mass Selective Detector (MSD) Mass Spectrometer (MS). The system may also be equipped with an additional detector, such as a Thermal Conductivity Detector (TCD). Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

The mass spectrometer will be configured to perform specific modes of ionization depending on which of the two types of ion sources is installed. If the electron impact (EI) ionization source is installed, only positive ion EI ionization analysis may be performed. However, if the chemical impact (CI) ionization source is installed, then either positive ion CI (PICI) or negative ion CI (NICI) analyses may be performed.

3 Equipment/Materials/Reagents

- a. Instrumentation – Agilent 7890 GC, 5975 MSD with EI or CI Source, TCD (if equipped), and MSD Chemstation software (or equivalent)
- b. Autosampler - Agilent ALS, CTC "Pal" Series, or Gerstel MPS automated sampler, accessories, and software (or equivalent)
- c. GC Column (MSD) - Agilent J&W DB-5 MS, 30 m, 0.25 mm i.d., 0.25 µm film (or equivalent)
- d. GC Column (TCD) - Agilent Molsieve, 30 m, 0.32 mm i.d., 12 µm film (or equivalent)
- e. Carrier Gas - Helium, 99.99% (high purity)
- f. CI Reagent Gas - Methane, 99.99% (high purity)
- g. Perfluorotributylamine (PFTBA, FC-43) (Agilent or equivalent)

- h. Perfluoro-5,8-dimethyl-3,6,9-trioxidodecane (PFDTD) Tuning Solution (Agilent or equivalent)
- i. Analytical balance
- j. Volumetric flask
- k. Autosampler vials – 2, 10 or 20 mL GC vials, crimp or screw top, with or without 100-500 µL inserts (Agilent or equivalent)
- l. Injection port liners - 4 mm split-splitless, tapered, with or without glass wool (Agilent or equivalent)
- m. Injection port septa - standard low-bleed 11 mm
- n. Autosampler syringes - Hamilton 701ASN 10 µL (or equivalent) for liquid injection and 1 mL or 2.5 mL Gerstel Headspace syringe or equivalent for headspace sampling
- o. Methanol (Optima Grade or equivalent)
- p. Caffeine Stock Standard (1 mg/mL):
A methanolic solution purchased from Cerilliant or other approved vendor. Stability and storage are determined by the manufacturer.
- q. Fentanyl Stock Standard (1 mg/mL):
A methanolic solution purchased from Cerilliant or other approved vendor. Stability and storage are determined by the manufacturer.
- r. MDEA Stock Standard (1 mg/mL):
A methanolic solution purchased from Cerilliant or other approved vendor. Stability and storage are determined by the manufacturer.
- s. Oxycodone Stock Standard (1 mg/mL):
A methanolic solution purchased from Cerilliant or other approved vendor. Stability and storage are determined by the manufacturer.
- t. Secobarbital Stock Standard (1 mg/mL):
A methanolic solution purchased from Cerilliant or other approved vendor. Stability and storage are determined by the manufacturer.
- u. Trazodone Stock Standard (1 mg/mL):
A methanolic solution purchased from Cerilliant or other approved vendor. Stability and storage are determined by the manufacturer.
- v. Formic acid (~89%, reagent grade)

- w. 0.05 M formic acid solution (GC/TCD performance check):
Dilute 215 μL of formic acid to 100 mL with deionized water in a graduated cylinder or flask. Mix well and store in glass at room temperature. Stable for at least one year.
- x. Concentrated Sulfuric Acid

4 Standards and Controls

4.1 PFTBA Tuning Solution

The PFTBA tuning solution is used for tuning the mass spectrometer and verifying mass assignment and accuracy when the EI source is installed. It is supplied by the instrument manufacturer and does not expire. It is stored in a glass container attached to the MSD. Under normal conditions, this should not need to be refilled.

4.2 PFDTD Tuning Solution

The PFDTD tuning solution is used for tuning the mass spectrometer and verifying mass assignment and accuracy when the CI source is installed. It is supplied by the instrument manufacturer and does not expire. It is stored in a glass container attached to the MSD. Under normal conditions, this should not need to be refilled.

4.3 Tox Testmix (10 $\mu\text{g/mL}$ each of Caffeine, Fentanyl, MDEA, Oxycodone, Secobarbital; 40 $\mu\text{g/mL}$ of Trazodone)

The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. To prepare: Pipet 250 μL each of Caffeine, Fentanyl, MDEA, Oxycodone, Secobarbital Stock Standards, and 1 mL Trazodone Stock Standard into a 25-mL volumetric flask. Bring to the mark with methanol and mix well. Store the solution in the refrigerator. It has a shelf-life of three years. This preparation may be appropriately scaled up.

4.4 TCD Testmix (Carbon Monoxide Performance Standard)

- a. To a 20 mL autosampler vial, add 1 mL of concentrated sulfuric acid.
- b. Add 50 μL of a 0.05 M formic acid solution.
- c. Immediately crimp-seal the autosampler vial and vortex for 10 seconds.
- d. Incubate autosampler vial at 100°C for 60 minutes in a laboratory heating block. Carbon Monoxide (CO) is produced quantitatively from the dehydration of formic acid in sulfuric acid.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks

The following steps will be performed daily, regardless of the ion source installed, mode of ionization, or the detector to be used. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Check to ensure that the GC wash vials are filled, the waste vials are empty, and all are in the appropriate positions.
- b. Record the remaining disk space on the hard drive. Use the Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- c. Record the line pressure of the building helium supply (carrier gas). The regulator should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the helium is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.

6.1.1 EI Source Daily Checks

If using the MSD with the EI source installed, perform the following steps:

- a. Check the Ion Gauge to ensure that there are no significant leaks in the system. Do not use if the source pressure is higher than EI analysis 6×10^{-5} torr.
- b. Perform a tune of the instrument. If Autotune (ATUNE) is selected, the mass spectrometer will tune itself using PFTBA. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, save and print the tune file (ATUNE) when completed.
- c. Perform an analysis of the Tox Testmix. Alternatively, if a specific analyte or analyte class is of interest, an appropriate testmix may be substituted. Open the appropriate testmix instrument method, and verify the parameters as listed in the 'Instrumental Conditions' section of this protocol. Set up a sequence, load the autosampler with a vial containing the Tox Testmix, and start the analysis. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC, RICs, and spectra for all six components in the Tox Testmix.
- d. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact

appropriate instrument support personnel.

6.1.2 CI Source Daily Checks

If using the MSD with the CI source installed, perform the following steps:

- a. Record the tank pressure of the methane tank (reagent gas). Change the tank if less than 100 p.s.i. remaining.
- b. Check the Ion Gauge to ensure that there are no significant leaks in the system. Do not use if the source pressure is higher than the following:
 - PICI analysis - 6×10^{-4} torr with reagent gas on at approximately 20%
 - NICI analysis - 6×10^{-4} torr with reagent gas on at approximately 40%
- c. Perform an analysis of the Tox Testmix. Alternatively, if a specific analyte or analyte class is of interest, an appropriate testmix may be substituted. Open the appropriate testmix instrument method, and verify the parameters as listed in the 'Instrumental Conditions' section of this protocol. Set up a sequence, load the autosampler with a vial containing the Tox Testmix, and start the analysis. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC, RICs, and spectra for all six components in the Tox Testmix.
- d. If sample analyses will be performed using negative ion mode, no additional daily checks will be required.
- f. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.1.3 TCD Daily Checks

If using the TCD, perform the following steps:

- a. Ensure a 1.0 or 2.5 mL Gerstel headspace syringe or equivalent is loaded into the autosampler.
- b. Ensure that the headspace autosampler injects into the back inlet of the GC.
- c. Check the status of the TCD using the front panel controls of the GC.
- d. Perform an analysis of the TCD Testmix. Open the appropriate testmix instrument method, and verify the parameters as listed in the 'Instrumental Conditions' section of this protocol. Set up a sequence, load the autosampler with a vial containing the TCD Testmix, and start the analysis. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the chromatogram.

- e. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.2 As Needed Checks

The following steps will be performed as needed based on system performance. Indicate completion in the appropriate QA/QC log.

- a. Replace the septum in the GC injection port.
- b. Replace the liner within the GC injection port.
- c. Check the syringe in the autosampler. Replace if needed.
- d. Replace the autosampler bands (if equipped).
- e. Perform positive and/or negative CI autotune.

7 Instrumental Conditions

7.1 Gas Chromatograph/Mass Spectrometer

7.1.1 Gas Chromatograph

Oven

Initial Temp:	60°C
Initial Time:	2.0 min
Ramp:	35°C/min
Final Temp:	280°C
Hold Time:	26.71 min

Inlet/Injector

Inj Vol:	1.0 µL
Mode:	Split
Split Ratio:	10:1
Inlet Temp:	220°C

Column

Type:	DB-5 MS
Length:	30 m
Diameter:	0.25 mm
Film Thickness:	0.25 µm
Mode:	Constant Flow
Init Flow:	1.2 mL/min

Average Lin Velocity: 40 cm/sec
Carrier Gas: Helium

7.1.2 Mass Spectrometer

Solvent Delay: 5.0 min
Scan Mode: Full Scan
Scan Range: 35-500 m/z

Temperatures

Same for EI, PICI, NICI

Transfer Line: 280°C
Source: 200°C
Quad: 150°C

7.2 Gas Chromatograph/Thermal Conductivity Detector

7.2.1 Autosampler

Oven temp: 60°C
Valve temp: 115°C
Transfer line temp: 115°C
Oven stabilization t: 0.1 min
Sample shaking rate: Low
GC cycle time: 4 min
Sample equil. time: 0.6 min
Vial pressurization t: 0.03 min
Loop fill time: 0.04 min
Loop equil. time: 0.02 min
Sample inj. time: 0.2 min

7.2.2 Gas Chromatograph

Oven

Temp: 40°C
Run time: 4 min
Equilibration time: 0.2 min

Inlet and Carrier

Inlet temp: 250°C
Injection mode: Split
Carrier gas: Helium
Carrier mode: Constant flow
Carrier flow: 13.2 mL/min
Split ratio: 3:1

Column

Type: Agilent-Molsieve
Length: 30 m
Internal diameter: 0.32 mm
Film thickness: 12 μ m

7.2.3 TCD

Temperature: 250°C
Reference flow: 20 mL/min
Makeup gas: Helium
Makeup flow: 2.5 mL/min

8 Decision Criteria

8.1 Autotune

If using the mass spectrometer, verify the results of the autotune. Compare the results of the autotune to previous autotune results. Significant voltage increases or changes in the isotope ratios indicate the need to initiate corrective maintenance procedures.

8.1.1 Electron Impact Ion Mode

The following are typical electron impact ion autotune values for the MSD:

- a. PFTBA Tune: Mass \pm 0.4 for m/z 69, 219, and 502
- b. Peak width: 0.45-0.65
- c. Relative abundance: 69 greater than 50%
219 greater than 50%
502 greater than 1%

8.1.2 Positive Ion Chemical Ionization Mode

The following are typical positive ion autotune (PCICH₄) values for the MSD:

- a. PFDTD Tune: Mass \pm 0.4 for m/z 41, 267, 599
- b. Peak width: 0.45-0.65
- c. Relative abundance: 69 present
267 present
599 present

8.1.3 Negative Ion Chemical Ionization Mode

The following are typical negative ion autotune (NCICH₄) values for the MSD:

- a. PFDTD Tune: Mass \pm 0.4 for m/z 185, 283, 351
- b. Peak width: 0.45-0.65
- c. Relative abundance: 185 present
283 present
351 present

8.2 Testmix

8.2.1 Tox Testmix

Verify the results of the Tox Testmix.

- a. In order for the instrument to be considered in good operating condition, all six components should generate well resolved, symmetrical peaks with baseline separation.
- b. A SNR of 3:1 will be the minimum response necessary to consider a response a peak.
- c. There should be no significant extraneous peaks in the chromatogram.
- d. The retention times of each component should be similar as compared to previous analyses (unless GC maintenance has been performed, such as column clipping or replacement).
- e. When analyzing the Tox Testmix, the following ions should be observed in the mass spectra of the RICs (in order of elution) and their mass assignments should be within \pm 0.5 m/z:

	<u>EI</u>	<u>PICI</u>
MDEA	72	208
Caffeine	194	195
Secobarbital	168	239
Oxycodone	315	316
Fentanyl	245	337
Trazodone	205	372

8.2.2 TCD Testmix

Verify the results of the TCD Testmix.

- a. The CO peak should be well separated from the nitrogen and oxygen peaks (>0.5 min baseline separation), and have a readily detectable peak.
- b. The GC column used in this procedure is a molecular sieve column, which may retain water. The column may be reconditioned by heating the GC oven to 225°C for >4 hours or overnight. Insufficient column conditioning results in poor chromatographic separation between the CO and air peaks.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Gas Chromatograph General Maintenance Protocol" (Inst 002) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual.*

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	10/01/12	New document titled "Performance Monitoring Protocol (QA/QC) for the Agilent GC/MS Prior to Analysis of Toxicological Samples." This document replaces Inst 111 and Inst 305.
1	10/04/18	Updated Section 1 Scope to include disciplines/categories of testing. Deleted Calibration section and renumbered. Updated heading on Section 5. Added 'appropriate instrument support personnel' to Sections 6.1 c, 6.1.1 d, 6.1.2 f, and 6.1.3 e. Added alternative testmix to Sections 6.1.1 c and 6.1.2 c. Updated Section 8.2.1 c & d to account for instrument variation and maintenance. Changed Section 8.2.1 e from 0.4 to 0.5 m/z. Updated 'Instrument Operation and Systems Support' in Section 13 and header.

Redacted - Signatures on File

Approval

Toxicology Technical
Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the JEOL AccuTOF DART

1 Scope

This document addresses the performance monitoring (QA/QC) of the JEOL AccuTOF DART. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, paint, explosives (chemistry), and Chemistry Unit general physical and chemical analysis.

2 Principle

This system consists of a JEOL AccuTOF Time-of-Flight (TOF) mass spectrometer (MS) and an IonSense Direct Analysis in Real Time Source (DART) ionization source. The TOF has the ability to measure mass accuracy with less than 5 milli-mass units (mmu) error over a range of 1 - 10,000 m/z. The DART source provides the capability of direct ionization with little to no sample preparation needed. The combined system may also be referred to as a 'DART.'

The TOF tube is sensitive to internal and external changes in temperature and humidity. These changes may result in a uniform shift of mass assignments. This shift is easily corrected by analyzing the calibrant in every sample analysis/data file and applying the new calibration algorithm, producing mass-corrected accurate mass data with less than 5 mmu error.

This performance monitoring protocol is based upon the manufacturer's recommendations. It should be noted that the instrument manufacturer can be referred to as either JEOL or IonSense. DART instrumentation and supplies are sold and supported by JEOL USA, Inc. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol" and the "Mass Spectrometer General Maintenance Protocol".

3 Equipment/Materials/Reagents

- a. Instrumentation - JEOL AccuTOF MS, IonSense DART Source, and data system with IonSense DART Controller and MassCenter Main software (or equivalent).
- b. Source Gas - Helium, 99.99% or greater purity (or equivalent)
- c. Source Gas - Nitrogen, 99.99% or greater purity (or equivalent)
- d. Capillary tubes
- e. Polyethylene Glycol (PEG), average molecular weight of 550 (Sigma or equivalent)

4 Standards and Controls

Neat Polyethylene Glycol (PEG) is used for performance verification and mass correction.

5 Calibration

The PEG is analyzed with every sample in the same data file, and used for fine mass correction. This results in the highest mass accuracy possible, taking all instrumental and environmental conditions into account at the time of analysis. This also ensures that calibration data will be available at any time in the future when data processing is performed. The operator is responsible for verifying that every calibration graph produced passes the 'Decision Criteria' section of this SOP. However, only the first calibration graph is printed and recorded.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Start Up

7.1.1 DART Initialization

- a. Open the DART Controller Software.
- b. Select 'Standby'.
- c. Select the appropriate operating temperature and gas type.
- d. Set the polarity required.
- e. Once DART has reached the operating temperature, select 'Run'.

7.1.2 TOF Initialization

- a. Open Mass Center Main.
- b. Open MS Tune Manager.
- c. Put instrument into 'Operate' mode.
- d. Load the appropriate tune file, verifying that the polarity matches the DART source.
- e. Verify that the voltage for Orifice 1 is set to 30 V (positive for positive ion mode,

negative for negative ion mode).

- f. Verify that the appropriate analyzer settings.

7.2 Daily Checks

- a. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 20 GB of free disk space. Do not use if less than 20 GB remain. If less than 20 GB of free disk space remain, contact appropriate instrument support personnel.
- b. Record the line pressure of the building nitrogen supply. The regulator should read at least 70 p.s.i. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the nitrogen is supplied by a gas cylinder, record the tank volume pressure. Change the tank if less than 100 p.s.i. remaining.
- c. Record the line pressure of the helium. The regulator should read at least 40 p.s.i. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the helium is supplied by a gas cylinder, record the tank volume pressure. Change the tank if less than 100 p.s.i. remaining.
- d. Check the vacuum pressure readings. The analyzer pressure must be below 9.9×10^{-5} Pa with the DART controller in standby (i.e., nitrogen flowing, not the helium).
- e. Perform a data acquisition for PEG using the procedure in 7.3 below. A PEG analysis from a sample acquisition collected the same day can be used as well.

7.3 Data Acquisition

- a. Open Spectrum Monitor. Ensure that there is a signal.
- b. Start an acquisition by selecting 'Acquire.' Type in the filename and comments and choose a data folder. Set the desired m/z range and verify the length of acquisition is appropriate (typically 2 minutes).
- c. Once the acquisition has started, ensure that there is a signal, and collect several seconds of background data.
- d. Dip the closed end of a glass capillary into the PEG, then place in the DART gas stream until a response is seen in the spectrum viewer for several seconds. Do this at least twice during the acquisition.

7.3.1 Time of Use Performance Verification/Mass Correction

Verify the performance of the instrument in the first data file acquired:

- a. Open the sample data file in the Chromatogram Viewer.

- b. View a background-subtracted PEG spectrum by holding down the 'shift' key and dragging across the PEG area with the right mouse button, then drag across a baseline area while holding the 'ctrl' key.
- c. Once the PEG spectrum appears in the Spectrum Viewer, generate a centroided spectrum.
- d. Right mouse click on the centroided spectrum, and make a calibration file from the spectrum.
- e. Select the appropriate polynomial order to provide the lowest residuals curve (1-R and 1-R* less than 10^{-10}).
- f. Evaluate the results using the 'Decision Criteria' section of this protocol.
- g. If the results are acceptable, print the PEG calibration graph.
- h. Save the calibration using an appropriate file name. The calibration file can now be applied to the sample chromatogram to obtain accurate mass data.

7.3.2 Data Correction

Verify the performance of the instrument in every data file acquired thereafter.

- a. The steps listed under 7.3.1 will be followed for every sample acquisition in order to ensure accurate mass results. However, it is not necessary to record subsequent results in the QA/QC log for the same day.
- b. Verify that the residuals (1-R and 1-R*) are 10^{-10} or lower before applying the calibration algorithm to the sample.

7.4 Instrument Shutdown

7.4.1 Time of Use Shutdown

- a. Put the MS Tune Manager in 'Analyzer HV' mode.
- b. Put the DART in 'Standby' mode, which will maintain the voltages and switch to nitrogen as the flow gas.
- c. Turn the heater off with nitrogen flowing to allow the heater to cool.

7.4.2 End of Day Shutdown

- a. Put the MS Tune Manager in 'Analyzer HV' mode.

- b. Turn the DART off, which will turn off the voltages and turn off the nitrogen flow gas.

7.4.3 As Needed Maintenance

- a. Replace the grid.
- b. Clean the cones, source, and source enclosure.

8 Instrumental Conditions

8.1 Mass Spectrometer

Ionization:	DART
Scan Mode:	Profile mode
Analyzer:	500 V peaks voltage for 50 - 500 m/z
	800 V peaks voltage for 80 – 800 m/z

9 Decision Criteria

9.1 PEG Performance Verification/Calibration

Verify that the residuals (1-R and 1-R*) are 10^{-10} or lower.

10 Calculations

Not applicable.

11 Measurement Uncertainty

Not applicable.

12 Limitations

Not applicable.

13 Safety

There are several areas of the MS which utilize extremely high voltage and vacuum conditions. For this reason, maintenance should only be performed with the system vented and the main

power off. Many MS components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

Take standard precautions for the handling of all chemicals, reagents, and standards. 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.

14 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
1	09/21/09	Removed hyphen in AccuTOF in title and sections 1 and 3a. Removed the word 'unique' from description of DART in section 2. In section 7.1.1c and d, changed heater temperature and needle voltage to values commonly used. Changed section 7.1.2e and f to clarify voltage polarity, and added the peaks voltage. Changed section 7.2a to increase the size of the hard drive disk space minimum. Re-worded section 7.2d to further clarify vacuum reading conditions. Changed section 7.3b to a fixed mass range. Re-worded section 7.3d to specify only PEG standard. Changed section 7.4.2b to more accurately describe the DART shutdown procedure. Added peaks voltage to section 8.1.
2	10/04/18	Updated Section 1 Scope to include disciplines/categories of testing. Updated Sections 7.1.1, 7.3.1, 7.4.1 and 7.4.2 for changes in latest software. Changed to 'appropriate analyzer settings' in Section 7.1.2 f. Added 'appropriate instrument support personnel' to Section 7.2 a, b & c. Added more detail to Section 7.3 d. Updated 'Instrument Operation and Systems Support' in Section 14 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Toxicology
Technical Leader:

Date: 09/28/2018

Paints and Polymers
Technical Leader:

Date: 09/28/2018

Explosives (Chemistry)
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

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Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Thermo LTQ LC/MS (ESI)

1 Scope

This document addresses the performance monitoring (QA/QC) of the Thermo LTQ LC/MS system consisting of a Thermo Electron LTQ Mass Spectrometer (MS) and a Liquid Chromatograph (LC). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, explosives (chemistry), and Chemistry Unit general physical and chemical analysis.

2 Principle

The LTQ system is comprised of a Shimadzu LC and a Thermo Electron Linear Ion Trap LTQ MS. The instrument is configured with an API source that is capable of electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). The instrument is primarily used in ESI mode. However, this protocol can also be used for APCI and APPI provided the method of ionization is clearly labeled in the resulting data and documentation. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Thermo Electron LTQ MS, API Source, Shimadzu Prominence LC, and Data System with XCalibur software (or equivalent)
- b. API Gas - Nitrogen, 99.99% (high purity or equivalent)
- c. Ion Trap Gas - Helium, 99.99% (high purity or equivalent)
- d. Methanol, Optima grade or equivalent
- e. Deionized Water, 18 MΩ·cm Milli-Q or equivalent
- f. Acetone, HPLC grade
- g. Ammonium Nitrate (NH₄NO₃), reagent grade
- h. Pierce ESI Positive Ion Calibration Solution (Thermo or equivalent)
- i. Caffeine (Sigma or equivalent)
- j. Pierce ESI Negative Ion Calibration Solution (Thermo or equivalent)

- k. Ammonium Hydroxide (NH₄OH), reagent grade
- l. Codeine (Sigma or equivalent)
- m. Brucine (Sigma or equivalent)
- n. Reserpine (Sigma or equivalent)
- o. γ -Aminobutyric Acid (GABA), (Sigma or equivalent)
- p. HMX, RDX, Tetryl, NG, PETN standards at 1000 μ g/mL (Cerilliant or equivalent)
- q. HMTD standard at 1000 μ g/mL (Cerilliant or equivalent)
- r. Volumetric glassware
- s. Infusion Syringe - 10 to 500 μ L LC syringe (Hamilton or equivalent)
- t. Basic LC Mobile Phase (95:5:0.03 Methanol:Water:Ammonium Hydroxide), or appropriate discipline specific mobile phase.
- u. 3.125 mM Ammonium Nitrate Mobile Phase (250 mg to 1 Liter water)
- v. C-18 Column (Grace Altima or equivalent)

4 Standards and Controls

4.1 Testmix (Toxicology/General Chemistry)

The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. Record all preparations in the Reagent Log. To prepare:

- a. Stock Solution - Weigh 1.5 mg GABA, 5.0 mg caffeine, 1.0 mg codeine, 1.0 mg brucine, and 1.0 mg reserpine into a 100-mL volumetric flask. Bring to the mark with methanol and mix well. Shelf life is three years when stored refrigerated in brown glass. This preparation may be appropriately scaled.
- b. Testmix Solution - Pipette 4.0 mL of the Stock Solution into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. Shelf life is three years when stored refrigerated in brown glass. This preparation may be appropriately scaled.

4.2 Testmix (Explosives Chemistry)

4.2.1 Explosives by ESI

The testmix is used to assess daily operating performance, mass assignment, and continued

integrity of the system. Record stock solution preparations in the Reagent Log. To prepare:

- a. Stock Solutions – place 1 mL of each 1000 µg/mL of HMX, RDX, Tetryl, NG and PETN standards in a separate 10 mL volumetric flask and dilute to the mark with acetone to achieve a final concentration of 100 µg/mL. Shelf life is two years when stored refrigerated in colored glass. This preparation may be appropriately scaled.
- b. Testmix Solution - Pipette 1 ml of each 100 µg/mL stock solution of HMX, RDX, Tetryl, NG, and PETN into a 10 mL volumetric flask and dilute to the mark with acetone to achieve a final concentration of 10 µg/mL. Shelf life is two years when stored refrigerated in colored glass. This preparation may be appropriately scaled.

4.2.2 Explosives by APCI

The HMTD Testmix is a 10 µg/mL solution of HMTD in deionized water. This solution must be prepared fresh each day. To prepare the testmix, add 10 µL of the 1000 µg/mL stock solution of HMTD to an autosampler vial, then add 990 µL of 18.2 MΩ·cm deionized water.

4.3 Calibration Solution

The calibration solution is used for coarse tuning and calibrating the mass spectrometer over the entire mass range. This procedure only needs to be performed when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

The calibration solution is purchased from Thermo Fisher Scientific or equivalent.

5 Calibration

The calibration procedure should be performed as needed, when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

- a. Load a 250 µL syringe with the appropriate calibration solution.
- b. Connect the infusion syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10 µL/minute.
- d. Load the tune file “esi_tune” (or equivalent).
- e. Check that instrument is in POSITIVE ION mode and collecting CENTROID data.
- f. Set the detector using the parameters listed in the 'Instrumental Conditions' section of

this protocol.

- g. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- h. Engage the ESI probe and turn on the MS.
- i. In Tune Plus, open the Calibrate dialog box, choose the 'Automatic' tab and check the individual tests or 'Select All' and then 'Start.'
- j. When the calibration is complete, it will display whether or not the calibration was successful.
 - If the procedure fails, repeat the calibration.
 - When the procedure passes, print the report and evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the spectrum of the calibration solution.
- k. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be performed.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Record the remaining disk space on the hard drive. Use either the Windows Explorer or Xcalibur program verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain. If analysis consists of multiple samples in a sequence, ensure that there is additional sufficient storage space.
- b. Record the line pressure of the building nitrogen supply (API gas). The regulator should read between 70 and 100 p.s.i. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the nitrogen is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 250 p.s.i. remaining.
- c. Record the line pressure of the building helium supply (ion trap gas). The regulator should read between 20 and 40 p.s.i. (30 – 60 p.s.i. if two instruments are to be run

off the same regulator). If it cannot maintain this pressure, contact appropriate instrument support personnel. If the helium is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.

- d. Check the Vacuum Pressure to ensure that no significant leaks are present in the system. Do not use if the Convectron Gauge reads above 2 torr, or the Ion Gauge (if present) reads above 20 microtorr.
- e. If using a Shimadzu LC System, prime each LC solvent line to be used that day. Open the prime valve on the front of each pump module to be used by turning the valve handle ninety degrees and press the "PURGE" button on the module. If the pump does not start priming, disengage remote control by pressing the "PUMP" button and then pressing the "PURGE" button again. After the prime cycle finishes (about three minutes), close the prime valves.
- f. If using the Shimadzu LC System, the autosampler solvent wash can be primed by pressing the "PURGE" button on the front of the autosampler module.
- g. Prepare the instrument for analysis of testmix. Verify that the instrument has the correct source probe installed (ESI), the correct tune file loaded (esi_tune, exp_tune or equivalent), positive ion or negative ion mode selected, and centroid data being collected.
- h. For Toxicology/General Chemistry Testmix: If a column is installed, remove it from that system and replace it with a zero-dead-volume union.
- i. For Toxicology/General Chemistry Testmix: Perform an analysis of the appropriate testmix prior to the analysis of case samples. For targeted analytes, a positive control can be substituted for the testmix. Use parameters listed in the 'Instrumental Conditions' section of this protocol. Select the appropriate mobile phase. Start the HPLC pump. Engage the ESI probe and turn on the MS. Start an acquisition using a filename such as 'TMyyymmdd' (or equivalent). Make three 5 μ L injections of the testmix solution at least 10 seconds apart by using the manual loop injector, and then stop the data collection. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC, RICs, and spectra for components in the testmix.
- j. For Explosives Testmix: Conduct a performance verification of the appropriate testmix through the column. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC, RICs, and spectra for components in the testmix.
- k. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

7.2 As Needed Checks

- a. Replace the metal needle as needed.
- b. Clean or replace the heated capillary as needed.

8 Instrumental Conditions

8.1 Testmix (Toxicology/General Chemistry)

Liquid Chromatograph

Mobile Phase:	Basic LC Mobile Phase (or discipline specific Mobile Phase)
Flow Rate:	0.3 mL/min
Column:	None
Inj Volume:	5 µL
Number of Inj:	3

Mass Spectrometer

Ionization:	ESI
Polarity:	Positive
Tune File:	esi_tune
Sheath Gas Flow:	6 (arb)
Aux Gas Flow:	3 (arb)
Sweep Gas Flow:	3 (arb)
Scan Mode:	Full Scan
Scan Range:	100-650 m/z

8.2 Testmix (Explosives Chemistry)

8.2.1 Explosives by ESI

Liquid Chromatograph

Mobile Phase:	60% Methanol : 40% 3.125 mM Ammonium Nitrate
Flow Rate:	0.3 mL/min
Column:	C-18
Inj Volume:	5 µL

Mass Spectrometer

Ionization:	ESI
Polarity:	Negative
Tune File:	exp_tune
Sheath Gas Flow:	20 (arb)
Aux Gas Flow:	5 (arb)
Sweep Gas Flow:	0 (arb)
Scan Mode:	Full Scan
Scan Range:	200-400 m/z (minimum)

8.2.2 Explosives by APCI

Liquid Chromatograph

Mobile Phase 1: Methanol with 1.25 mM Ammonium Nitrate
Mobile Phase 2: DI H₂O with 1.25 mM Ammonium Nitrate
Flow Rate: 0.3 mL/min
Gradient: 0-2 min 90% Mobile Phase 2
12-14 min 50% Mobile Phase 2
17-20 min 90% Mobile Phase 2
Column: C-18
Inj Volume: 10 µL

Mass Spectrometer

Ionization: APCI
Polarity: Positive
Tune File: HMTD_TUNE (or equivalent)
Sheath Gas Flow: 35 (arb)
Aux Gas Flow: 15 (arb)
Sweep Gas Flow: 0 (arb)
Scan Mode: Full Scan
Scan Range: 150-250 m/z (minimum)

8.3 Calibration

Mass Spectrometer

Ionization: ESI
Tune File: esi_tune
Scan Mode: Full Scan
Scan Range: 100-2000 m/z

9 Decision Criteria

9.1 Testmix (Toxicology/General Chemistry)

Verify the results of the testmix. The following ions should be observed in the three testmix injections: RICs should show contemporaneous signals for components at the following masses:

- Caffeine 195 m/z
- Codeine 300 m/z
- Brucine 395 m/z
- Reserpine 609 m/z

9.2 Testmix (Explosives Chemistry)

9.2.1 Explosives by ESI

Verify the results of the testmix. RICs should show contemporaneous signals for components at the following masses:

- HMX (+NO₃) 358 m/z
- RDX (+NO₃) 284 m/z
- Tetryl (+NO₃) 349 m/z
- NG (+NO₃) 289 m/z
- PETN (+NO₃) 378 m/z

9.2.2 Explosives by APCI

Verify the results of the testmix. RICs should show contemporaneous signals for components at the following masses:

- HMTD 224, 209, 207 m/z

9.3 Calibration

Verify the results of the calibration. The calibration will indicate if the procedure was successful. For reference, the individual ions for the calibration solution are:

- Caffeine 195 m/z
- MRFA 524 m/z
- Ultramark 1022 m/z
1122 m/z
1222 m/z
1322 m/z
1422 m/z
1522 m/z
1622 m/z
1722 m/z
1822 m/z
1922 m/z

10 Calculations

Not applicable.

11 Measurement Uncertainty

Not applicable.

12 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

14 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

"Preparation of Chemical Reagents" (Tox 103) *Toxicology SOP Manual*.

"Solid Phase Extraction of Opioids from Biologicals with Analysis by LC-Tandem MS" (Tox 418) *Toxicology SOP Manual*.

FBI Laboratory Safety Manual.

Rev #	Issue Date	History
2	04/25/16	Updated section 3 to include standards for Explosive Chemistry, calibration solutions, as well as mobile phase and column selection. Removed Ultramark 1621, MFRA, acetonitrile, ammonium formate, formic acid, and acetic acid. Explosives Chemistry Testmix added to section 4.2. Removed preparation of calibration solution in section 4.3. Differentiation made between testmix procedure for Toxicology/General Chemistry and Explosives Chemistry, section 7.1. Section 7.2 removed capillary tubing. Explosives Chemistry Instrument conditions placed in section 8.2. Section 9.2 updated for Explosives Chemistry Decision Criteria.
3	04/29/16	Section 4.2 fixed typo and added that preparations may be appropriately scaled. Changed wording in section 7.1 to allow Windows Explorer to be used to check disk space. Section 8.2 corrected for Mobile Phase concentrations.
4	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Changed Section 3 t to 250 mg. Added HMTD to Section 3 q. Changed 'all' to 'stock solution' in Section 4.2. Added 'Explosives by ESI' for clarification in Sections 4.2.1, 8.2.1, and 9.2.1. Added Sections 4.2.2, 8.2.2, and 9.2.2 for 'Explosives by APCI' and HMTD Testmix. Updated heading in Section 6. Added 'appropriate instrument support personnel' to Sections 5 k and 7.1 b, c, & k. Added targeted analytes to Section 7.1 j. Updated 'Instrument Operation and Systems Support' in Section 14 and header. Added '(minimum)' to scan range in 8.2.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Toxicology
Technical Leader:

Date: 09/28/2018

Explosives (Chemistry)
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Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Redacted - Signatures on File

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the AB Sciex QTRAP LC/MS

1 Scope

This document addresses the performance monitoring (QA/QC) of the AB Sciex QTRAP LC/MS system consisting of an AB Sciex QTRAP Mass Spectrometer (MS) and a Liquid Chromatograph (LC). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Toxicology.

2 Principle

The QTRAP system is comprised of a Shimadzu LC and an AB Sciex QTRAP MS that can be used as a Triple-Stage Quadrupole or as a Linear Ion Trap (in MS or MS/MS mode). The instrument is configured with an Atmospheric Pressure Ionization (API) source that is capable of both electrospray (ESI) and atmospheric pressure chemical (APCI) ionization. Currently, the instrument is primarily used as a Triple-Stage Quadrupole in the ESI mode. However, this protocol can also be used for APCI provided the method of ionization is clearly labeled in the resulting data and documentation. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation – AB Sciex QTRAP MS, API Source, Shimadzu LC, and data system with “Analyst” software (or equivalent)
- b. API Gas - Nitrogen, 99.99% (high purity or equivalent)
- c. Collision Gas & Ion Trap Gas - Nitrogen, 99.999% (high purity or equivalent)
- d. Methanol, HPLC grade or equivalent
- e. Deionized Water, 18 MΩ·cm Milli-Q or equivalent
- f. Optima Grade Water or equivalent
- g. Acetonitrile, HPLC grade or equivalent
- h. Formic Acid - ~98% (Fluka or equivalent)
- i. AB Sciex PPG (Polypropylene Glycol) 3000 (include in Part # 401936)
- j. AB Sciex Mass Spectrometer Solution Kit (Part # 401936)

- k. Caffeine (Sigma or equivalent)
- l. Agilent ESI Tuning Mix
- m. Ammonium Acetate, reagent grade
- n. Codeine (Sigma or equivalent)
- o. Brucine (Sigma or equivalent)
- p. Reserpine (Sigma or equivalent)
- q. Volumetric glassware
- r. Infusion Syringe - 10 to 500 μ L LC syringe (Hamilton or equivalent)

4 Standards and Controls

4.1 Testmix

The stock testmix is prepared by weighing 5.0 mg caffeine, 1.0 mg codeine, 1.0 mg brucine, and 1.0 mg reserpine into a 100-mL volumetric flask. Bring to the mark with methanol and mix well. The testmix is further diluted by using 50 μ L of the stock testmix and diluted to 10-mL volumetric flask with methanol. Store the solution in the refrigerator. It has a shelf-life of three years. The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system.

4.2 Calibration Solutions

The calibration solution is used for coarse tuning and calibrating the mass spectrometer over the entire mass range. Using this instrument as a Triple-Stage Quadrupole, requires it to be calibrated both in the positive and negative mode. As a result, two calibration solutions are needed to calibrate this instrument.

4.2.1 Preparation of PPG Dilution Solvent

This solution can be purchased directly from AB Sciex or prepared as follows:

- a. Dissolve 15.4 milligrams of ammonium acetate in 49.9 mL of deionized water.
- b. It is essential to dissolve the ammonium acetate in deionized water first.
- c. To 49.9 mL of methanol, add 0.1 mL of formic acid and 0.1 mL of acetonitrile.

- d. Mix the two solutions together to make the final PPG Dilution Solvent. Store the solution in the refrigerator. It has a shelf-life of three years.

4.2.2 Preparation of Diluted PPG Standard Solution (Positive Mode)

The Diluted PPG Standard Solution is used to calibrate the Q1 (Quadrupole) and Q3 in the positive mode. The PPG Standard (in Mass Spectrometer Solution Kit) purchased from AB Sciex is diluted with the prepared PPG Dilution Solvent (listed above) or the one supplied from the purchase kit at a ratio of 1:50.

- a. Put 20 mL of the PPG Dilution Solvent in a clean vial and remove 0.4 mL leaving 19.6 mL.
- b. Add 0.4 mL of PPG Standard ($1 \times 10^{-4}\text{M}$) to the 19.6 mL of PPG Dilution Solvent and mix well. Store the solution in the refrigerator. It has a shelf-life of three years.

4.2.3 PPG 3000 Standard (Negative Mode)

The PPG 3000 Standard is used to calibrate the Q1 (Quadrupole) and Q3 in the negative mode. The standard is purchased directly from AB Sciex and no dilution is needed.

5 Calibration

The calibration procedure should be performed as needed, when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

5.1 Calibration of Q1 Quadrupole in the Positive Mode

- a. Load a 250 μL syringe with the Diluted PPG Standard.
- b. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10 $\mu\text{L}/\text{minute}$.
- d. Enter the 'Manual Tuning' window.
- e. Load the tune file 'Q1 Pos PPGs.dam' reference file.
- f. Check that instrument is in POSITIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
 - Correct tune file is loaded.
 - 'PPGs Pos' is loaded under the dialog box label 'Standard'.

- Q1 and Unit are checked under 'Quad/Resolution'.
 - Search range is set to 3 amu with threshold at 200 cps (counts per seconds) under the 'Peak Search Parameter'.
 - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA (Multi-Channel Averaging) mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- l. Save the calibration and print the calibration report.

5.2 Calibration of Q3 Quadrupole in the Positive Mode

- a. Load a 250 µL syringe with the Diluted PPG Standard.
- b. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10 µL/minute.
- d. Enter the 'Manual Tuning' window.
- e. Load the tune file 'Q3 Pos PPGs.dam' reference file.
- f. Check that instrument is in POSITIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
- Correct tune file is loaded.
 - 'PPGs Pos' is loaded under the dialog box label 'Standard'.
 - Q1 and Unit are checked under 'Quad/Resolution'.
 - Search range is set to 3 amu with threshold at 200 cps (counts per seconds) under the 'Peak Search Parameter'.
 - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.

- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- l. Save the calibration and print the calibration report.

5.3 Calibration of Q1 Quadrupole in the Negative Mode

- a. Load a 250 μ L syringe with the PPG 3000 standard.
- b. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10 μ L/minute.
- d. Enter the 'Manual Tuning' window.
- e. Load the tune file 'Q1 Neg PPGs.dam' reference file.
- f. Check that instrument is in NEGATIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
 - Correct tune file is loaded.
 - 'PPGs Neg' is loaded under the dialog box label 'Standard'.
 - Q1 and Unit are checked under 'Quad/Resolution' section.
 - Search range is set to 3 amu with threshold at 200 cps under the 'Peak Search Parameter' section.
 - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.

- l. Save the calibration and print the calibration report.

5.4 Calibration of Q3 Quadrupole in the Negative Mode

- a. Load a 250 μ L syringe with the PPG 3000 standard.
- b. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10 μ L/minute.
- d. Enter the 'Manual Tuning' window.
- e. Load the tune file 'Q3 Neg PPGs.dam' reference file.
- f. Check that instrument is in NEGATIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
 - Correct tune file is loaded.
 - 'PPGs Neg' is loaded under the dialog box label 'Standard'.
 - Q1 and Unit are checked under 'Quad/Resolution' section.
 - Search range is set to 3 amu with threshold at 200 cps under the 'Peak Search Parameter' section.
 - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- m. Save the calibration and print the calibration report.

5.5 Completion of Calibration

If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be performed.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 1GB of free disk space. Do not use if less than 1GB remain.
- b. Record the line pressure of the building nitrogen supply (API gas). The regulator should read between 60 and 100 p.s.i. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the nitrogen is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.
- c. Verify that the system is under vacuum and the ion gauge is reading less than 6.0×10^{-5} torr.
- d. Prepare the instrument for analysis of the testmix. Verify that the instrument has the correct source probe installed (ESI), positive ion mode selected, and centroid data being collected.
- e. Perform an analysis of the testmix prior to the analysis of samples using parameters listed in the 'Instrumental Conditions' section of this protocol. Start the LC pump. Engage the ESI probe and turn on the MS. Start an acquisition using a filename such as 'testmix' (or equivalent). Make three 5 μ L injections of the testmix solution approximately 10 seconds apart by using the manual loop injector, and then stop the data collection. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC and extracted ions for all components in the testmix.
- f. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

7.2 As Needed Checks

- a. Replace heaters.

- b. Clean or replace the heated capillary.

8 Instrumental Conditions

8.1 Testmix (Positive Mode)

Liquid Chromatograph

Mobile Phase:	From discipline specific SOP
Flow Rate:	0.3 mL/min
Column:	None
Inj Volume:	5 µL
Number of Inj:	3

Mass Spectrometer

Ionization:	ESI
Scan Mode:	SIM
Source Temp:	600°C
Mass:	95, 300, 395 and 609 m/z

9 Decision Criteria

9.1 Testmix

Verify the results of the testmix. The following ions should be observed in the three testmix injections:

- Caffeine 195 m/z
- Codeine 300 m/z
- Brucine 395 m/z
- Reserpine 609 m/z

9.2 Calibration

Verify the results of the calibration. The calibration will indicate if the procedure was successful. Calibration in the positive mode for Q1 and Q3, confirms the presence of ions m/z 59.1, 175.1, 616.5, 906.7, 1254.9, 1545.1, 2010.5, and 2242.6. In addition, verify that the sensitivity and peak width are within range as specified below.

Positive Mode Q1 and Q3

	Sensitivity (cps) m/z 906.7	Peak width (amu)	Sensitivity (cps) m/z 2242	Peak width (amu)
Q1	> 2.0e7	0.6 – 0.8	> 1.0e6	0.6 – 0.8
Q3	> 2.0e7	0.6 – 0.8	> 8.0e5	0.6 – 0.8

Calibration in the negative mode for Q1 and Q3, confirms the presence of ions m/z 45.0, 585.4, 933.6, 1223.8, 1572.1, 1863.3, 2037.4, and 2211.6. In addition, verify that the sensitivity and peak width are within range as specified below.

Negative Mode Q1 and Q3

	Sensitivity (cps) m/z 933.6	Peak width (amu)	Sensitivity (cps) m/z 2037.4	Peak width (amu)
Q1	> 2.0e7	0.6 – 0.8	3.0e6	0.6 – 0.8
Q3	> 1.0e7	0.6 – 0.8	N/A	0.6 – 0.8

10 Calculations

Not applicable.

11 Measurement Uncertainty

Not applicable.

12 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

14 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	12/14/07	New document.
1	03/14/12	Updated instrument manufacturer to 'AB Sciex' in Title and Sections 1, 2, 3, 4.2.1, 4.2.2 and 4.2.3. Removed ammonium hydroxide and added Optima grade water to Section 3. Changed testmix dilution in Section 4.1. Added storage and stability information to Section 4.2.1.d. Added molar concentration in Section 4.2.2.b. Removed the word "case" from Section 7.1e. Changed scan mode to SIM, added source temperature and masses to Section 8.1. Changed scan mode to MRM and added mass reference to Section 8.2. Added instrument manual to Section 14.
2	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Removed 'Spark Holland Symbiosis from Sections 1 & 2 and added Shimadzu LC to Section 2. Added 'appropriate instrument support personnel' to Sections 5.5 and 7.1 b & f. Removed 'Manufacturer's Maintenance Agreement' Section 7.3. Updated heading in Section 6. Changed to discipline specific SOP in Section 8.1. Removed Section 8.2 (covered in Section 5). Updated 'Instrument Operation and Systems Support' in Section 14 and header.

Approval

Redacted - Signatures on File

Toxicology Technical
Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Thermo LTQ OrbiTrap XL LC/MS (ESI)

1 Scope

This document addresses the performance monitoring (QA/QC) of the Thermo LTQ OrbiTrap XL LC/MS system consisting of a Thermo Electron LTQ OrbiTrap XL Mass Spectrometer (MS), and a Liquid Chromatograph (LC). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, and Chemistry Unit general physical and chemical analysis.

2 Principle

The LTQ OrbiTrap XL system is comprised of a Shimadzu Liquid Chromatograph (LC) and a Thermo Electron Linear Ion Trap LTQ MS coupled to an OrbiTrap XL High Resolution MS. This system can be used for both unit mass and high resolution accurate mass chemical analyses. The instrument is configured with an API source that is capable of electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). The instrument is primarily used in ESI mode. However, this protocol can also be used for APCI and APPI provided the method of ionization is clearly labeled in the resulting data and documentation. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Thermo Electron LTQ MS, OrbiTrap XL, API Source, Shimadzu Prominence LC, and Data System with XCalibur software (or equivalent)
- b. API Gas - Nitrogen, 99.99% (high purity or equivalent)
- c. Ion Trap Gas - Helium, 99.99% (high purity or equivalent)
- d. Methanol, Optima grade or equivalent
- e. Deionized Water, 18 M Ω ·cm Milli-Q or equivalent
- f. Acetonitrile, HPLC grade or equivalent
- g. Ammonium Formate, reagent grade
- h. Formic Acid, reagent grade
- i. LTQ ESI Positive Ion Calibration Solution (Thermo or equivalent)

- j. LTQ ESI Negative Ion Calibration Solution (Thermo or equivalent)
- k. Ammonium Hydroxide (NH₄OH), reagent grade
- l. Codeine (Sigma or equivalent)
- m. Brucine (Sigma or equivalent)
- n. Reserpine (Sigma or equivalent)
- o. γ -Aminobutyric Acid (GABA), (Sigma or equivalent)
- p. Volumetric glassware
- q. Infusion Syringe - 10 to 500 μ L LC syringe (Hamilton or equivalent)

4 Standards and Controls

4.1 Testmix

The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. Record all preparations in the Reagent Log. To prepare:

- a. Stock Solution - Weigh 1.5 mg GABA, 5.0 mg caffeine, 1.0 mg codeine, 1.0 mg brucine, and 1.0 mg reserpine into a 100-mL volumetric flask. Bring to the mark with methanol and mix well. Shelf life is three years when stored refrigerated in brown glass. This preparation may be appropriately scaled up.
- b. Testmix Solution - Pipet 4.0 mL of the Stock Solution into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. Shelf life is three years when stored refrigerated in brown glass. This preparation may be appropriately scaled up.

4.2 Calibration Solution

The calibration solution is used for coarse tuning and calibrating both the LTQ and the Orbitrap XL over the entire mass range. This procedure only needs to be performed when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

The calibration solution is purchased from Thermo Fisher Scientific or equivalent.

5 Calibration

The calibration procedure should be performed as needed, when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

- a. Load a 250 μ L syringe with the appropriate calibration solution.
- b. Connect the infusion syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10 μ L/minute.
- d. Load the tune file "calibration_solution_positive" (or equivalent).
- e. Check that instrument is in POSITIVE ION mode and collecting CENTROID data.
- f. Set the detector using the parameters listed in the 'Instrumental Conditions' section of this protocol.
- g. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- h. Engage the ESI probe and turn on the MS.
- i. To perform an accurate mass calibration for the OrbiTrap only, open the 'Calibrate' dialog box in Tune Plus, choose the 'Semi-Automatic' tab and check **ONLY** 'Mass Calibration' for FT and then 'Start.'
- j. To perform a unit mass calibration for the LTQ only, open the 'Calibrate' dialog box in Tune Plus, choose the 'Semi-Automatic' tab and check **ONLY** 'Select All - Ion Trap' and then 'Start.'
- k. When the calibration is complete, it will display whether or not the calibration was successful.
 - If the procedure fails, repeat the calibration.
 - When the procedure passes, print the report and evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the spectrum of the calibration solution.
- l. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be performed.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Record the remaining disk space on the hard drive. Use the Xcalibur program (menu: actions > check disk space) to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain. If analysis consists of multiple samples in a sequence, ensure that there is additional sufficient storage space.
- b. Record the line pressure of the building nitrogen supply (API gas). The regulator should read between 70 and 100 p.s.i. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the nitrogen is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 250 p.s.i. remaining.
- c. Record the line pressure of the building helium supply (ion trap gas). The regulator should read between 20 and 40 p.s.i. (30 – 60 p.s.i. if two instruments will be run off the same regulator). If it cannot maintain this pressure, contact appropriate instrument support personnel. If the helium is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.
- d. Check the oil level in the vacuum pumps housed in the compartment directly below the LTQ. If a significant amount of oil is present in the mist filter, then ballast pumps individually by temporarily rotating the ballast switch until the oil has been evacuated.
- e. Check the vacuum pressure to ensure that no significant leaks are present in the system. Do not use if the convectron gauge reads above 2 torr, or the ion gauge (if present) reads above 20 microtorr.
- f. Prime each LC solvent line to be used that day. Open the prime valve on the front of each pump module to be used by turning the valve handle ninety degrees and press the PURGE button on the module. If the pump does not start priming, disengage remote control by pressing the PUMP button and then pressing the PURGE button again. After the prime cycle finishes (about three minutes), close the prime valves.
- g. Prime the autosampler solvent wash by pressing the PURGE button on the front of the autosampler module, then rinse the autosampler needle by pressing the RINSE button.

- h. Verify that the instrument has the correct source probe installed (ESI), the correct tune file loaded (testmix_pos or equivalent), positive ion mode selected, and centroid data being collected. If a column is installed, remove it from that system and replace it with a zero-dead-volume union.
- i. Select the proper analyzer. In Tune Plus, select 'Ion Trap' analyzer for unit mass resolution using the LTQ. For accurate mass analysis using the Orbitrap XL, select 'FTMS' analyzer and a resolution of 60,000.
- j. Perform an analysis of the testmix prior to the analysis of case samples. For targeted analytes, a positive control can be substituted for the testmix. For testmix analysis, use parameters listed in the 'Instrumental Conditions' section of this protocol. Start the HPLC pump. Engage the ESI probe and turn on the MS. Start an acquisition using a filename such as 'TMymmdd' (or equivalent). Make three 5 μ L injections of the testmix solution at least 10 seconds apart by using the manual loop injector, and then stop the data collection. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC and spectra for components in the testmix.
- k. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

7.2 As Needed Checks

- a. Re-cut or replace the sample capillary as needed.
- b. Clean or replace the heated capillary as needed.
- c. Clean the ion sweep cone (the heated interface front plate) as needed.

8 Instrumental Conditions

8.1 Testmix

Liquid Chromatograph

Mobile Phase:	From discipline specific SOP
Flow Rate:	0.3 mL/min
Column:	None
Inj Volume:	5 μ L

Mass Spectrometer

Ionization:	ESI
Tune File:	testmix_pos
Sheath Gas Flow:	14 (arb)
Aux Gas Flow:	3 (arb)

Sweep Gas Flow: 3 (arb)
 Scan Mode: Full Scan
 Scan Range: 100-650 m/z

8.2 Calibration

Mass Spectrometer

Ionization: ESI
 Tune File: calibration_solution_positive
 Scan Mode: Full Scan
 Scan Range: 100-2000 m/z

9 Decision Criteria

9.1 Testmix: Unit Mass

Verify the results of the testmix. The following ions should be observed in the three testmix injections when using the system for unit mass resolution. RICs should show contemporaneous signals for components at the following masses:

Caffeine	195 m/z
Codeine	300 m/z
Brucine	395 m/z
Reserpine	609 m/z

9.2 Testmix: Accurate Mass

When using the OrbiTrap analyzer for accurate mass analysis, the testmix components should be observed within ± 3 mmu of their expected monoisotopic masses:

	<u>Formula</u>	<u>Expected Mass</u>	<u>Acceptable Mass Range</u>
Caffeine	C ₈ H ₁₁ O ₂ N ₄	195.0877	195.0847 - 195.0907
Codeine	C ₁₈ H ₂₂ O ₃ N	300.1594	300.1564 - 300.1624
Brucine	C ₂₃ H ₂₇ O ₄ N ₂	395.1965	395.1935 - 395.1995
Reserpine	C ₃₃ H ₄₁ O ₉ N ₂	609.2807	609.2777 - 609.2837

9.3 Calibration

Verify the results of the calibration. The calibration will indicate if the procedure was successful. For reference, the individual ions for the calibration solution are:

Caffeine	195 m/z
MRFA	524 m/z
Ultramark	1022 m/z
	1122 m/z
	1222 m/z

1322 m/z
1422 m/z
1522 m/z
1622 m/z
1722 m/z
1822 m/z
1922 m/z

10 Calculations

Not applicable.

11 Measurement Uncertainty

Not applicable.

12 Limitations

This procedure is specific to positive-ion analyses. The LTQ OrbiTrap XL system should not be switched to negative ion mode without the assistance of appropriate instrument support personnel.

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

14 References

Manufacturer's Instrument Manuals for the specific models and accessories used (electronic or hardcopy).

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and*

Systems Support SOP Manual.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual.*

"Preparation of Chemical Reagents" (Tox 103) *Toxicology SOP Manual.*

"Solid Phase Extraction of Opioids from Biologicals with Analysis by LC-Tandem MS" (Tox 418) *Toxicology SOP Manual.*

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	07/06/09	New document
1	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Removed Waters Alliance LC from Sections 2 and 3 a. Added 'appropriate instrument support personnel' to Sections 5 l, 7.1 b, c & k, and 12. Updated heading in Section 6. Added targeted analytes to Section 7.1 j. Changed to discipline specific SOP in Section 8.1. Reduced decimal places from five to four in Section 9.2. Updated 'Instrument Operation and Systems Support' in Section 14 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Toxicology
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

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Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the AB Sciex API Triple-Stage Quadrupole LC/MS

1 Scope

This document addresses the performance monitoring (QA/QC) of the AB Sciex Triple-Stage Quadrupole Liquid Chromatograph/Mass Spectrometer (LC/MS) system consisting of an API Triple-Stage Quadrupole MS and a Shimadzu LC. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Toxicology.

2 Principle

The API system is comprised of a Shimadzu LC and an API Triple-Stage Quadrupole (in MS or MS/MS mode). The instrument is configured with an Atmospheric Pressure Ionization (API) source that is capable of both electrospray (ESI) and atmospheric pressure chemical ionization (APCI). Currently, the instrument is primarily used as a Triple-Stage Quadrupole in the ESI mode. However, this protocol can also be used for APCI provided the method of ionization is clearly labeled in the resulting data and documentation. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation – API Triple-Stage MS, API Source, Shimadzu LC, and data system with “Analyst” software (or equivalent)
- b. API Gas - Nitrogen, 99.99% (high purity or equivalent)
- c. Collision Gas & Ion Trap Gas - Nitrogen, 99.999% (high purity or equivalent)
- d. Methanol, HPLC grade or equivalent
- e. Deionized Water, 18 MΩ·cm Milli-Q or equivalent
- f. Acetonitrile, HPLC grade or equivalent
- g. Formic Acid - ~98% (Fluka or equivalent)
- h. AB Sciex PPG (Polypropylene Glycol) 3000 (included in Part # 401936)
- i. AB Sciex Mass Spectrometer Solution Kit (Part # 401936)
- j. Caffeine (Sigma or equivalent)

- k. Agilent ESI Tuning Mix
- l. Ammonium Acetate, reagent grade
- m. Codeine (Sigma or equivalent)
- n. Brucine (Sigma or equivalent)
- o. Reserpine (Sigma or equivalent)
- p. Volumetric glassware
- q. Infusion Syringe - 10 to 500 μ L LC syringe (Hamilton or equivalent)

4 Standards and Controls

4.1 Testmix

The stock testmix is prepared by weighing 5.0 mg caffeine, 1.0 mg codeine, 1.0 mg brucine, and 1.0 mg reserpine into a 100-mL volumetric flask. Bring to the mark with methanol and mix well. The testmix is further diluted by using 50 μ L of the stock testmix and diluted to 10-mL volumetric flask with methanol. Store the solution in the refrigerator. It has a shelf-life of three years. The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system.

4.2 Calibration Solutions

The calibration solution is used for coarse tuning and calibrating the mass spectrometer over the entire mass range. Using this instrument requires it to be calibrated both in the positive and negative mode. As a result, two calibration solutions are needed to calibrate this instrument.

4.2.1 Preparation of Positive PPG Dilution Solvent

This solution can be purchased directly from AB Sciex or prepared as follows:

- a. Dissolve 15.4 milligrams of ammonium acetate in 49.9 mL of deionized water.
- b. To 49.9 mL of methanol, add 0.1 mL of formic acid and 0.1 mL of acetonitrile.
- c. Mix the two solutions together to make the Positive PPG Dilution Solvent. Store the solution in the refrigerator. It has a shelf-life of three years.

4.2.2 Preparation of Negative PPG Dilution Solvent

This solution can be purchased directly from AB Sciex or prepared as follows:

- a. Dissolve 385.0 milligrams of ammonium acetate in 49.9 mL of deionized water.
- b. To 49.9 mL of methanol, add 0.1 mL of formic acid and 0.1 mL of acetonitrile.
- c. Mix the two solutions together to make the Negative PPG Dilution Solvent. Store the solution in the refrigerator. It has a shelf-life of three years.

4.2.3 Preparation of Diluted PPG Standard Solution (Positive Mode)

The Diluted PPG Standard Solution is used to calibrate the Q1 (Quadrupole) and Q3 in the positive mode. The PPG Standard (in Mass Spectrometer Solution Kit) purchased from AB Sciex is diluted with the prepared positive PPG Dilution Solvent (see section 4.2.1) or the one supplied from the purchase kit at a ratio of 1:500 to produce a solution that is $2.0 \times 10^{-7}\text{M}$.

- a. Add 1.0 mL of PPG Standard ($1 \times 10^{-4}\text{M}$) to 49.0 mL of the positive PPG Dilution Solvent and mix. This produces a $2 \times 10^{-6}\text{M}$ solution.
- b. Add 2.0 mL of $2 \times 10^{-6}\text{M}$ solution to 18.0 mL of the positive PPG Dilution Solvent and mix. This produces a solution that is $2 \times 10^{-7}\text{M}$. Store the solution in the refrigerator. It has a shelf-life of three years.

4.2.4 PPG 3000 Standard Solution (Negative Mode)

The PPG 3000 Standard ($3.0 \times 10^{-5}\text{M}$) is used to calibrate the Q1 (Quadrupole) and Q3 in the negative mode. The PPG 3000 Standard purchased directly from AB Sciex is diluted with the prepared negative PPG Dilution Solvent (see section 4.2.2) or the one supplied from the purchase kit at a ratio of 1:10 to produce a solution that is $3.0 \times 10^{-5}\text{M}$.

- a. Add 2.0 mL of PPG 3000 Standard to 18.0 mL of the negative PPG Dilution Solvent and mix. This produces a $3 \times 10^{-5}\text{M}$ solution. Store the solution in the refrigerator. It has a shelf-life of three years.

5 Calibration

The calibration procedure should be performed as needed, when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

5.1 Calibration of Q1 Quadrupole in the Positive Mode

- a. Load a 250 μL syringe with the Diluted PPG Standard Solution (see section 4.2.3).
- b. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10 $\mu\text{L}/\text{minute}$.

- d. Enter the 'Manual Tuning' window (Refer to API LC/MSMS System Installation Guide and API LC/MS/MS System Operator's manual).
- e. Load the tune file 'Q1 Pos PPGs.dam' reference file.
- f. Check that instrument is in POSITIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
 - Correct tune file is loaded.
 - 'PPGs Pos' is loaded under the dialog box label 'Standard'.
 - Q1 and Unit are checked under 'Quad/Resolution'.
 - Search range is set to 3 amu with threshold at 200 cps (counts per seconds) under the 'Peak Search Parameter'.
 - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA (Multi-Channel Averaging) mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- l. Mass calibrate the mass spectrometer using the procedure defined in the instrument manuals referenced in this document.
- m. Save the calibration and print the calibration report.

5.2 Calibration of Q3 Quadrupole in the Positive Mode

- a. Load a 250 μ L syringe with the Diluted PPG Standard Solution (see section 4.2.3).
- b. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10 μ L/minute.
- d. Enter the 'Manual Tuning' window (Refer to API LC/MS/MS System Installation Guide and API LC/MS/MS System Operator's manual).
- e. Load the tune file 'Q3 Pos PPGs.dam' reference file.

- f. Check that instrument is in POSITIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
 - Correct tune file is loaded.
 - 'PPGs Pos' is loaded under the dialog box label 'Standard'.
 - Q1 and Unit are checked under 'Quad/Resolution'.
 - Search range is set to 3 amu with threshold at 200 cps (counts per seconds) under the 'Peak Search Parameter'.
 - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- l. Mass calibrate the mass spectrometer using the procedure defined in the instrument manuals referenced in this document.
- m. Save the calibration and print the calibration report.

5.3 Calibration of Q1 Quadrupole in the Negative Mode

- a. Load a 250 μ L syringe with the PPG 3000 Standard Solution (section 4.2.4).
- b. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10 μ L/minute.
- d. Enter the 'Manual Tuning' window (Refer to API LC/MS/MS System Installation Guide and API LC/MS/MS System Operator's manual).
- e. Load the tune file 'Q1 Neg PPGs.dam' reference file.
- f. Check that instrument is in NEGATIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
 - Correct tune file is loaded.
 - 'PPGs Neg' is loaded under the dialog box label 'Standard'.

- Q1 and Unit are checked under 'Quad/Resolution' section.
 - Search range is set to 3 amu with threshold at 200 cps under the 'Peak Search Parameter' section.
 - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- l. Mass calibrate the mass spectrometer using the procedure defined in the instrument manuals referenced in this document.
- m. Save the calibration and print the calibration report.

5.4 Calibration of Q3 Quadrupole in the Negative Mode

- a. Load a 250 μ L syringe with the PPG 3000 Standard Solution (section 4.2.4).
- b. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 10 μ L/minute.
- d. Enter the 'Manual Tuning' window (Refer to API LC/MS/MS System Installation Guide and API LC/MS/MS System Operator's manual).
- e. Load the tune file 'Q3 Neg PPGs.dam' reference file.
- f. Check that instrument is in NEGATIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
- Correct tune file is loaded.
 - 'PPGs Neg' is loaded under the dialog box label 'Standard'.
 - Q1 and Unit are checked under 'Quad/Resolution' section.
 - Search range is set to 3 amu with threshold at 200 cps under the 'Peak Search Parameter' section.
 - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.

- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- l. Mass calibrate the mass spectrometer using the procedure defined in the instrument manuals referenced in this document.
- m. Save the calibration and print the calibration report.

5.5 Completion of Calibration

If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be performed.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 1GB of free disk space. Do not use if less than 1 GB remain.
- b. Record the line pressure of the building nitrogen supply (API gas). The regulator should read between 60 and 100 p.s.i. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the nitrogen is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.
- c. Verify that the system is under vacuum and the ion gauge is reading less than 6.0×10^{-5} torr.

- d. Prepare the instrument for analysis of the testmix. Verify that the instrument has the correct source probe installed (ESI), positive ion mode selected, and centroid data is being collected.
- e. Perform an analysis of the testmix prior to the analysis of samples using parameters listed in the 'Instrumental Conditions' section of this protocol. Start the HPLC pump. Engage the ESI probe and turn on the MS. Start an acquisition using a filename such as 'testmix' (or equivalent). Make three 2 μ L injections of the testmix solution approximately 10 seconds apart by using the manual loop injector, and then stop the data collection. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the combined ion chromatogram and extracted ions for all components in the testmix.
- f. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

7.2 As Needed Checks

- a. Replace temperature heaters.
- b. Clean or replace the heated capillary.

7.3 Manufacturer's Maintenance Agreement

Calibration and repair will be performed as needed. Under the maintenance agreement with AB Sciex, the manufacturer will conduct an annual preventative maintenance.

8 Instrumental Conditions

8.1 Testmix (Positive Mode)

Liquid Chromatograph

Mobile Phase:	From discipline specific SOP
Flow Rate:	0.3 mL/min
Column:	None
Inj Volume:	2 μ L
Number of Inj:	3

Mass Spectrometer

Ionization:	ESI
Scan Mode:	SIM
Source Temp:	600°C
Mass:	95, 300, 395 and 609 m/z

8.2 Calibration

Mass Spectrometer

Ionization: ESI
Scan Mode: MRM
Scan Range: Manufacturer's Performance Specifications in the instrument manuals.

9 Decision Criteria

9.1 Testmix

Verify the results of the testmix. The following ions should be observed in the three testmix injections:

- Caffeine 195 m/z
- Codeine 300 m/z
- Brucine 395 m/z
- Reserpine 609 m/z

9.2 Calibration

Verify the results of the calibration. The calibration will indicate if the procedure was successful. Calibration in the positive mode for Q1 and Q3, confirms the presence of ions m/z 59.1, 175.1, 500.4, 616.5, 906.7, 1080.8 and 1196.9. In addition, verify that the sensitivity and peak width are within range as specified below.

Positive Mode Q1 and Q3

	Sensitivity (cps) m/z 906.7	Peak width (amu)
Q1	> 1.5e7	0.6 – 0.8
Q3	> 1.3e7	0.6 – 0.8

Calibration in the negative mode for Q1 and Q3, confirms the presence of ions m/z 45.0, 411.3, 585.4, 933.6 and 1223.8. In addition, verify that the sensitivity and peak width are within range as specified below.

Negative Mode Q1 and Q3

	Sensitivity (cps) m/z 933.6	Peak width (amu)
Q1	> 2.0e7	0.6 – 0.8
Q3	> 1.0e7	0.6 – 0.8

10 Calculations

Not applicable.

11 Measurement Uncertainty

Not applicable.

12 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

14 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	03/14/12	New document.
1	09/10/13	Removed Spark Holland Symbiosis LC from Sections 1,2, and 3. Removed Optima Grade Water, Mobile Phase A, and Mobile Phase B from Section 3. Removed 5000 from Sections 5.1.d, 5.2.d, 5.3.d, and 5.4.d. Changed mobile phase in Section 8.1. Added API 6500 System Installation guide and System Operator's manual to Section 14.
2	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Added 'appropriate instrument support personnel' to Sections 5.5 and 7.1 b & f. Updated heading in Section 6. Changed subunit to discipline in Section 8.1. Updated Instrument Operation and Systems Support in Section 14 and header.

Approval

Redacted - Signatures on File

Toxicology Technical
Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Thermo Exactive OrbiTrap LC/MS (ESI)

1 Scope

This document addresses the performance monitoring (QA/QC) of the Thermo Exactive OrbiTrap LC/MS system consisting of a Thermo Electron Exactive OrbiTrap Mass Spectrometer (MS), and a Liquid Chromatograph (LC). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, explosives (chemistry), and Chemistry Unit general physical and chemical analysis.

2 Principle

The Exactive OrbiTrap system is comprised of a Waters LC and a Thermo Electron Exactive OrbiTrap High Resolution MS. This system can be used for high resolution accurate mass chemical analyses. The instrument is configured with an atmospheric pressure ionization (API) source that is capable of electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). The instrument is primarily used in ESI mode. However, this protocol can also be used for APCI and APPI provided the method of ionization is clearly labeled in the resulting data. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Thermo Electron Exactive OrbiTrap MS, API Source, Waters Acquity UPLC (or equivalent), and Data System with Xcalibur software (or equivalent)
- b. API Gas - Nitrogen, 99.99% (high purity or equivalent)
- c. Caffeine (Sigma or equivalent)
- d. Codeine (Sigma or equivalent)
- e. Brucine (Sigma or equivalent)
- f. Reserpine (Sigma or equivalent)
- g. γ -Aminobutyric Acid (GABA), (Sigma or equivalent)
- h. Optima Grade Methanol (or equivalent)
- i. LTQ Velos OrbiTrap ESI Positive Ion Calibration Solution (Thermo or equivalent)

- j. LTQ ESI Negative Ion Calibration Solution (Thermo or equivalent)
- k. Infusion Syringe - 10 to 500 μ L LC syringe (Hamilton or equivalent)
- l. Deionized Water, 18 M Ω ·cm Milli-Q or equivalent
- m. Acetone, HPLC grade
- n. Volumetric glassware
- o. Ammonium Nitrate (NH₄NO₃), reagent grade
- p. HMX, RDX, Tetryl, NG, PETN standards at 1000 μ g/mL (Cerilliant or equivalent)
- q. 3.125 mM Ammonium Nitrate Mobile Phase (250 mg to 1 Liter water)
- r. Waters Cortecs UPLC C18 1.6 μ m, 2.1 mm X 50 mm, or equivalent

4 Standards and Controls

4.1 Testmix (Toxicology/General Chemistry)

The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. Record all preparations in the Reagent Log. To prepare:

- a. Stock Solution - Weigh 1.5 mg GABA, 5.0 mg caffeine, 1.0 mg codeine, 1.0 mg brucine, and 1.0 mg reserpine into a 100-mL volumetric flask. Bring to the mark with methanol and mix well. Shelf life is three years when stored refrigerated in brown glass. This preparation may be appropriately scaled.
- b. Testmix Solution - Pipet 4.0 mL of the Stock Solution into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. Shelf life is three years when stored refrigerated in brown glass. This preparation may be appropriately scaled.

4.2 Testmix (Explosives Chemistry)

The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. Record stock solution preparations in the Reagent Log. To prepare:

- a. 100 μ g/mL Stock Solution - Pipette 1 mL of each 1000 μ g/mL of HMX, RDX, Tetryl, NG and PETN standards in a separate 10 mL volumetric flask and dilute to the mark with acetone to achieve a final concentration of 100 μ g/mL. Shelf life is two years when stored refrigerated in colored glass. This preparation may be appropriately scaled.

- b. 10 µg/mL Stock Solution - Pipette 1 mL of each 100 µg/mL stock solution of HMX, RDX, Teteryl, NG, and PETN into a 10 mL volumetric flask and dilute to the mark with acetone to achieve a concentration of 10 µg/mL. Shelf life is two years when stored refrigerated in colored glass. This preparation may be appropriately scaled.
- c. Testmix Solution - For daily use, dilute 20 µL of the 10 µg/mL stock solution to 1 mL with a 50:50 solution of methanol/water.

4.3 Calibration Solution

The calibration solution is used for coarse tuning and calibrating the mass spectrometer over the entire mass range. This procedure only needs to be performed when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

The calibration solution is purchased from Thermo Fisher Scientific or equivalent.

5 Calibration

5.1 Calibration (Positive Mode)

- a. Load an infusion syringe with the LTQ Velos OrbiTrap ESI positive ion calibration solution.
- b. Connect the infusion syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 5 µL/minute.
- d. On the tune page click “Calibrate” and confirm that MS Mass Calibration (pos) is checked.
- e. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- f. Engage the ESI probe and turn on the MS.
- g. Click the “Calibrate” button to start the calibration.
- h. When the calibration is complete, it will display whether or not the calibration was successful. If the procedure fails, repeat the calibration.
- i. If all requirements are within specification, prepare records as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective action to be taken.

5.2 Calibration (Negative Mode)

- a. Load an infusion syringe with the LTQ ESI negative ion calibration solution.
- b. Connect the infusion syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump rate to 5 μ L/minute.
- d. On the tune page click "Calibrate" and confirm that MS Mass Calibration (neg) is checked.
- e. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- f. Engage the ESI probe and turn on the MS.
- g. Click the "Calibrate" button to start the calibration.
- h. When the calibration is complete, it will display whether or not the calibration was successful. If the procedure fails, repeat the calibration.
- i. If all requirements are within specification, prepare records as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be performed.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Record the remaining disk space on the hard drive. Use either the Windows Explorer or Xcalibur program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain. If analysis consists of multiple samples in a sequence, ensure that there is additional sufficient storage space.
- b. Record the line pressure of the building nitrogen supply (API gas). The regulator should read between 70 and 100 p.s.i. If it cannot maintain this pressure, contact

appropriate instrument support personnel. If the nitrogen is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 250 p.s.i. remaining.

- c. Check the oil level of the vacuum pump.
- d. Check the vacuum pressure under instrument status on the tune page. If a green circle with a white check mark in it is present, the system is ready.
- e. To prime LC system:
 - 1. Open up the Acquity UPLC Console and select Acquity UPLC system in the menu on the left side of the screen.
 - 2. From the control drop down menu select system start up.
 - 3. Confirm for the sample manager (SM) that the strong wash, weak wash, and sample syringe are checked and 3 is entered in the cycles box.
 - 4. Confirm for the binary solvent manager (BSM) that all the boxes are checked and that the duration time is set to 5 minutes.
 - 5. Click the start button to start priming the system.
- f. For Toxicology/General Chemistry Testmix: If a column is installed, remove it from that system and replace it with a zero-dead-volume union.
- g. For Toxicology/General Chemistry Testmix: Perform an analysis of the appropriate testmix prior to the analysis of case samples. For targeted analytes, a positive control can be substituted for the testmix. Use parameters listed in the 'Instrumental Conditions' section of this protocol. Select the appropriate mobile phase. Start the HPLC pump. Engage the ESI probe and turn on the MS. Start an acquisition using a filename such as 'TMyyymmdd' (or equivalent). Make three 5 μ L injections of the testmix solution at least 10 seconds apart by using the manual loop injector, and then stop the data collection. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC, RICs, and spectra for components in the testmix.
- h. For Explosives Chemical Analysis: Conduct a performance verification of the appropriate testmix through the column. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC, RICs, and spectra for components in the testmix.
- i. If all requirements are within specification, prepare records as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

7.2 As Needed Checks

- a. Replace the metal needle as needed.
- b. Clean or replace the heated capillary as needed.
- c. Clean the ion sweep cone (the heated interface front plate) as needed.

8 Instrumental Conditions

8.1 Testmix (Toxicology/General Chemistry)

Liquid Chromatograph

Mobile Phase:	From discipline-specific SOP
Flow Rate:	0.15 mL/min
Column:	None
Inj Volume:	5 µL

Mass Spectrometer

Ionization:	ESI
Tune File:	testmix_pos
Sheath Gas Flow:	9 (arb)
Aux Gas Flow:	3 (arb)
Sweep Gas Flow:	0 (arb)
Scan Mode:	Full Scan
Scan Range:	100-650 m/z
Resolution:	75000

8.2 Testmix (Explosives Chemistry)

Liquid Chromatograph

Mobile Phase:	From discipline-specific SOP
Flow Rate:	0.5 mL/min
Column:	Waters Cortecs UPLC C18 1.6 µm, 2.1 mm X 50 mm
Inj Volume:	8 µL

Mass Spectrometer

Ionization:	ESI
Tune File:	exp_tune
Sheath Gas Flow:	20 (arb)
Aux Gas Flow:	5 (arb)
Sweep Gas Flow:	0 (arb)
Scan Mode:	Full Scan
Scan Range:	200-400 m/z (minimum)
Resolution:	17500

8.3 Calibration

Mass Spectrometer

Ionization: ESI
Scan Mode: Full Scan
Scan Range: 100-2000 m/z

9 Decision Criteria

9.1 Testmix (Toxicology/General Chemistry)

When using the OrbiTrap analyzer for accurate mass analysis, the testmix components should be observed within the range below from their expected monoisotopic masses:

	<u>Formula</u>	<u>Expected Mass</u>	<u>Acceptable Mass Range</u>
Caffeine	C ₈ H ₁₁ O ₂ N ₄	195.0877	195.0847 - 195.0907
Codeine	C ₁₈ H ₂₂ O ₃ N	300.1594	300.1564 - 300.1624
Brucine	C ₂₃ H ₂₇ O ₄ N ₂	395.1965	395.1935 - 395.1995
Reserpine	C ₃₃ H ₄₁ O ₉ N ₂	609.2807	609.2777 - 609.2837

9.2 Testmix (Explosives Chemistry)

When using the OrbiTrap analyzer for accurate mass analysis, the testmix components should be observed within the range below from their expected monoisotopic masses:

	<u>Formula</u>	<u>Expected Mass</u>	<u>Acceptable Mass Range</u>
HMX(+NO ₃)	C ₄ H ₈ O ₁₁ N ₉	358.0338	358.0288-358.0388
RDX(+NO ₃)	C ₃ H ₆ O ₉ N ₇	284.0222	284.0172-284.0272
Tetry(+NO ₃)	C ₇ H ₅ O ₁₁ N ₆	349.0011	348.9961-349.0061
NG(+NO ₃)	C ₃ H ₅ O ₁₂ N ₄	288.9898	288.9848-288.9948
PETN(+NO ₃)	C ₅ H ₈ O ₁₅ N ₅	378.0011	377.9961-378.0061

9.3 Calibration (Positive Mode)

Verify the results of the calibration. The calibration will indicate if the procedure was successful. For reference, the individual ions for the calibration solution are:

Caffeine	195 m/z
MRFA	524 m/z
Ultramark	1022 m/z
	1122 m/z
	1222 m/z
	1322 m/z
	1422 m/z
	1522 m/z
	1622 m/z
	1722 m/z

1822 m/z
1922 m/z

9.4 Calibration (Negative Mode)

Verify the results of the calibration. The calibration will indicate if the procedure was successful. For reference, the individual ions for the calibration solution are:

Sodium dodecyl sulfate	265 m/z
Sodium taurocholate	517 m/z
Ultramark	1280 m/z
	1380 m/z
	1480 m/z
	1580 m/z
	1680 m/z
	1780 m/z

10 Calculations

Not applicable.

11 Measurement Uncertainty

Not applicable.

12 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

14 References

Manufacturer's Instrument Manuals for the specific models and accessories used (electronic or hardcopy).

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual.*

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and Systems Support SOP Manual.*

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual.*

"Preparation of Chemical Reagents" (Tox 103) *Toxicology SOP Manual.*

"Solid Phase Extraction of Opioids from Biologicals with Analysis by LC-Tandem MS" (Tox 418) *Toxicology SOP Manual.*

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
2	05/23/17	Changed 'Stock Solution' to '100 µg/mL Stock Solution' in 4.2.a. Changed 'Testmix Solution' to '10 µg/mL Stock Solution' in 4.2.b. Added 'Testmix Solution' section 4.2.c, dilution for daily use.
3	10/04/18	Updated Section 1 Scope to include disciplines/categories of testing. Changed Section 3 q to 250 mg. Changed 'all' to 'stock solution' in Section 4.2. Added 'appropriate instrument support personnel' to Sections 5.1 i. 5.2 I, and 7.1 b & i. Updated heading in Section 6. Changed 'subunit' to 'discipline' mobile phase in section 8.1. Added '(minimum)' to scan range in 8.2. Updated mobile phase and flow rate in section 8.2. Reduced decimal places from five to four in Section 9.1. Corrected typos in Section 9.2. Updated 'Instrument Operation and Systems Support' in Section 14 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Toxicology
Technical Leader:

Date: 09/28/2018

Explosives (Chemistry)
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Agilent 7890 GC/ECD/NPD

1 Scope

This document addresses the performance monitoring (QA/QC) of the Agilent 7890 GC/ECD/NPD System. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, and Chemistry Unit general physical and chemical analysis.

2 Principle

The Agilent 7890 GC/ECD/NPD is a gas chromatograph (GC) with two injectors, two columns, and two internal detectors. The instrument is configured with two capillary columns, each leading to their respective detectors. The column in the front position is connected to an electron capture detector (ECD), and the column in the back leads to a nitrogen-phosphorus detector (NPD). Samples are introduced into either column through the use of standard GC injection ports. The system may also be referred to as the 'ECD/NPD.'

This performance monitoring protocol is based upon the manufacturer's recommendations. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Agilent 7890 Gas Chromatograph, Electron Capture Detector, Nitrogen-Phosphorus Detector, and Chemstation Software (or equivalent)
- b. Autosampler - Agilent or CTC "Pal" Series automated sampler, accessories, and software (or equivalent)
- c. ECD GC Column - Rtx-ClPest (Restek or equivalent)
- d. NPD GC Column - Rtx-1701 (Restek or equivalent)
- e. Carrier Gas - Helium, 99.99% (high purity)
- f. Detector Makeup Gas - Nitrogen, 99.99% (high purity)
- g. Hydrogen gas - high purity or equivalent
- h. Compressed air
- i. Autosampler vials - 2 mL GC vials, crimp or screw top, with or without 200 µL

inserts (HP or equivalent)

- j. Injection port liners - 4 mm split-splitless, tapered, with or without glass wool (HP or equivalent)
- k. Injection port septa - low-bleed 11 mm (HP or equivalent)
- l. Autosampler syringes - Hamilton 701ASN 10 μ L (or equivalent)

4 Standards and Controls

4.1 Performance Verification Standards

- a. Organochlorine (OC) Pesticides Stock Solution:
A hexane:toluene (1:1) solution approximately 1 mg/mL each of aldrin, 4,4'-DDT, endrin, endrin aldehyde, and lindane. Purchased as a special order item from Chemservice, Inc. Store refrigerated in glass. Stable for at least two years, or as determined by manufacturer.
- b. ECD Pesticides Testmix Solution:
Dilute 25 μ L of the OC pesticides stock solution to 50 mL with hexane, yielding a solution approximately 0.5 μ g/mL in each component. Store refrigerated in glass. Stable for at least two years. A portion of this testmix is analyzed prior to each batch of GC-ECD analyses.
- c. Organophosphate (OP) Pesticides Stock Solution:
A hexane solution approximately 1 mg/mL each of chlorpyrifos, diazinon, fenchlorphos, parathion (ethyl), and prophos. Purchased as a special order item from Chemservice, Inc. Store refrigerated in glass. Stable for at least two years, or as determined by manufacturer.
- d. NPD Pesticides Testmix Solution:
Dilute 500 μ L of the OP pesticides stock solution to 25 mL in hexane, yielding a solution approximately 20 μ g/mL in each component. Store refrigerated in glass. Stable for at least two years. A portion of this testmix is analyzed prior to each batch of GC-NPD analyses.
- e. The performance verification standard is used to assess daily operating performance and continued integrity of the gas chromatography-detector system. The positive control standard for the applicable discipline-specific procedure and detector will be analyzed and evaluated as the ECD or NPD Performance Standard prior to the analysis of evidence.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks

The following steps will be performed daily prior to use. Enter the appropriate information in the QA/QC log.

- a. Check to ensure that the GC wash vials are filled, the waste vials are empty, and all are in the appropriate positions.
- b. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- c. Record the line pressure of the building helium supply (carrier gas). The regulator should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the instrument is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. is remaining.
- d. Record the line pressure of the building nitrogen supply (makeup gas). The regulator should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the instrument is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. is remaining.
- e. Conduct a performance verification standard analysis and evaluate prior to the analysis of evidence.
- f. Evaluate the results using the 'Decision Criteria' section of this SOP. If the results are acceptable, print the chromatogram for the performance verification standard.
- g. Prepare specific documentation as outlined in the "General Instrument Maintenance Protocol."

6.2 As Needed Checks

The following steps will be performed as needed based on system performance. Record the appropriate information on the QA/QC log.

- a. Replace the septum in the GC injection port.
- b. Replace the liner within the GC injection port.

- c. Check the GC syringe in the autosampler. Replace if needed.
- d. Check the internal bungee cords in the autosampler. Replace if needed.
- e. Check the plungers in each autosampler syringe. Replace if needed.

7 Instrumental Conditions

7.1 Gas Chromatograph/Nitrogen-Phosphorus Detector

Oven

Temp 1:	125°C
Hold 1:	1 min
Ramp 1:	7°C/min
Temp 2:	280°C
Hold 2:	22 min

Column

Type:	Rtx-1701
Length:	30 m
Inner diameter:	0.32 mm
Film thickness:	0.5 µm

Inlet and Carrier

Inlet temp:	250°C
Injection mode:	Split
Carrier gas:	Helium
Carrier mode:	Constant pressure
Carrier pressure:	13.39 psi
Split ratio:	15:1

NPD

Temperature:	250°C
Offset:	10
Makeup flow: (nitrogen)	30 mL/min
Air flow:	60 mL/min
Hydrogen flow:	2 mL/min

7.2 Gas Chromatograph/Electron Capture Detector

Oven

Temp 1	125°C
Hold 1	1 min
Ramp 1	7°C/min
Temp 2	280°C
Hold 2	22 min

Column

Type	Rtx-CIPest
Length	30 m
Inner diameter	0.32 mm
Film thickness	0.5 µm

Inlet and Carrier

Inlet temp	230°C
Injection mode	Splitless
Carrier gas	Helium
Carrier mode	Constant pressure
Carrier pressure	16.85 psi
Splitless time	0.5 min

ECD

Temp	300°C
Makeup gas	Nitrogen
Makeup flow	30 mL/min

8 Decision Criteria

8.1 Performance Verification Standard

The peaks of the analytes in the performance standard should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. Peak areas and retention times should compare favorably to previous analyses of the performance standard.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform such duties involved in the maintenance or troubleshooting of this instrument.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

13 References

Manufacturer's Instrument Manuals for specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Gas Chromatograph General Maintenance Protocol" (Inst 002) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	06/21/06	New document which replaces original titled "Performance Monitoring Protocol (QA/QC) for the HP 6890 GC/ECD/NPD"
1	06/08/09	Changed model number in Title and Sections 1, 2, and 3.a. Removed references to Hewlett Packard and HP in the Title and Sections 1 and 2. Clarified statement in Section 7.2 and added c and d. Changed 'slit' to 'split' in Section 8.1. Made changes to oven parameters in Sections 8.1 and 8.2. Made changes to solutions in Section 4.1 to reflect Tox Subunit modifications.
2	xxxx/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Deleted Calibration Section and renumbered. Updated heading in Section 5. Changed 'subunit' to 'discipline' in Section 4.1 e. Added 'appropriate instrument support personnel' to Section 7.1 c & d. Updated 'Instrument Operation and Systems Support' in Section 14 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Toxicology
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Agilent 7890 High Temperature GC/FID

1 Scope

This document addresses the performance monitoring (QA/QC) of the Agilent 7890 High Temperature GC/FID. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Explosives (chemistry), fire debris, and Chemistry Unit general physical and chemical analysis.

2 Principle

The Agilent 7890 High Temperature GC/FID is a gas chromatograph (GC) with a Multi-Mode inlet and a Flame Ionization Detector (FID). 'High Temperature' refers to the fact that the inlet is programmable to increase during a run and attain a higher temperature than an inlet is normally set, and the capillary column used is specifically designed to allow the temperature to be raised to a higher level than is typically applied to GC columns. A sample is introduced onto the column by either manual or autosampler injection through an injection port.

This performance monitoring protocol is generally based upon the manufacturer's recommendations. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Agilent 7890 Gas Chromatograph, Flame Ionization Detector, Multi-Mode inlet, and Chemstation Software (or equivalent)
- b. Autosampler - Agilent or CTC "Pal" Series automated sampler, accessories, and software (or equivalent)
- c. GC Column - Zebron "Inferno" ZB-1HT Capillary Column, 15 m x 0.25 mm, 0.1 µm film thickness (Phenomenex or equivalent)
- d. Carrier Gas - Helium, 99.99% (high purity)
- e. Compressed air (from air purifier, compressor, tank, or equivalent)
- f. Hydrogen Gas, 99.9% (from gas generator, tank, or equivalent)
- g. Nitrogen Gas, 99.99% (from gas generator, tank, or equivalent)
- h. Hexane or Cyclohexane (B&J UV grade or equivalent)

- i. n-Paraffin Mix ~ C₁₆-C₄₄ at 0.01% (w/w) in cyclohexane (Supelco or equivalent)
- j. Injection port liners - 4 mm split-splitless, tapered, with or without glass wool (HP or equivalent)
- k. Injection port septa - low-bleed 11 mm (HP or equivalent)
- l. Syringe - Hamilton 701ASN 10 µL (or equivalent)

4 Standards and Controls

4.1 Hi-Temp GC Testmix

The testmix is a commercially available hexane or cyclohexane solution of n-alkanes made up of ~ C₁₆-C₄₄. This performance standard should have a concentration of approximately 0.001% (v/v) paraffin in hexane/cyclohexane and be stored at room temperature in an amber volumetric flask. This solution has a shelf-life of three years. The testmix is used to verify daily operating performance and continued integrity of the gas chromatograph-detector system. It will be analyzed and evaluated prior to the analysis of evidence.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log.

- a. Check to ensure that the GC wash vials are filled, the waste vials are empty, and all are in the appropriate positions.
- b. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- c. Record the line pressure of the building helium supply (carrier gas). The regulator should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the instrument is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.

- d. Ensure that the hydrogen generator is on and supplying gas to the FID. Verify that the FID is lit.
- e. Load the testmix method (see 'Instrumental Conditions' section). Program the autosampler to inject 1 μ L of the testmix. It is also acceptable to manually inject 1 μ L of the testmix and press *Start* on the GC.
- f. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the chromatogram for the performance verification standard.
- g. Prepare specific documentation as outlined in the "General Instrument Maintenance Protocol."

6.2 As Needed Checks

The following steps are to be performed as needed. Record the appropriate information in the QA/QC log.

- a. Replace the liner within the GC injection port.
- b. Check the GC syringe in the autosampler. Replace if needed.

7 Instrumental Conditions

Inlet/Injector

Inj Vol:	1.0 μ L
Mode:	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
Ramp1:	30°C/min
Final Temp1:	100°C
Ramp1 Time:	0 min
Ramp2:	15°C/min
Final Temp2:	400°C
Ramp2 Time:	3.5 min
Run Time:	27.0 min
Equil Time:	0.5 min

Column

Type: Zebron ZB-1HT (or equivalent)
Length: 15 m
Diameter: 0.25 mm
Film Thickness: 0.1 μ m
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: 30.0 mL/min
Makeup Gas: Nitrogen

8 Decision Criteria

8.1 Testmix

Verify the results of the testmix.

- a. In order for the instrument to be considered in good operating condition, all components should generate well-resolved, Gaussian-shaped peaks with baseline separation.
- b. A SNR of 3:1 will be the minimum response necessary to consider a response a peak.
- c. There should be no extraneous peaks in the testmix chromatogram greater than 5% of the tallest peak.
- d. The retention times of components should not deviate by $\pm 3\%$ compared to previous runs of the testmix.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Not applicable.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Gas Chromatograph General Maintenance Protocol" (Inst 002) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
3	04/01/11	Make-up gas for FID changed from helium to nitrogen to reflect current instrument set-up in sections 3 and 8. Specified commercial nature of n-alkane mixture in section 4.1. Changed the testmix injection volume from 2 µL to 1 µL in section 8. Specified current testmix concentrations in sections 3 and 4.1.
4	04/25/16	Sections 2 and 3 updated to reflect use of Agilent Multi-Mode Inlet
5	10/04/18	Updated Section 1 Scope to include disciplines/categories of testing. Removed PTV inlet from Sections 2 & 3 a. Added injection port liners and septa, Section 3 j & k. Updated heading in Section 5. Added 'appropriate instrument support personnel' to Section 6.1 c. Added Section 6.1 d. Updated 'Instrument Operation and Systems Support' in Section 13 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader:

09/28/2018

Fire Debris Technical
Leader:

Date: 09/28/2018

Explosives (Chemistry
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

Issuance

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Agilent GC/ECD

1 Scope

This document addresses the performance monitoring (QA/QC) of the Agilent GC/ECD system. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Explosives (chemistry).

2 Principle

The Agilent GC/ECD system consists of a gas chromatograph (GC) with an Electron Capture Detector (ECD). This performance monitoring protocol is generally based upon the manufacturer's recommendations. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Agilent 7890 GC, Electron Capture Detector, and Chemstation software (or equivalent)
- b. Autosampler - Agilent ALS, accessories, and software (or equivalent)
- c. GC Column - Agilent DB-5 MS, 6 m, 0.25 mm i.d., 0.25 μ m film (or equivalent)
- d. Carrier Gas - Helium, 99.99% (high purity)
- e. Nitrogen Gas, 99.99% (from gas generator, tank, or equivalent)
- f. Cyclotrimethylene trinitramine (RDX), 1000 μ g/mL (Cerilliant or equivalent)
- g. Cyclotetramethylene tetranitramine (HMX), 1000 μ g/mL (Cerilliant or equivalent)
- h. Pentaerythritol tetranitrate (PETN), 1000 μ g/mL (Cerilliant or equivalent)
- i. Nitroglycerin (NG), 1000 μ g/mL (Cerilliant or equivalent)
- j. Trinitrotoluene (TNT), 1000 μ g/mL (Cerilliant or equivalent)
- k. Ethylene glycol dinitrate (EGDN), 100 μ g/mL (Cerilliant or equivalent)
- l. 2,4-Dinitrotoluene (2,4-DNT), 1000 μ g/mL (Cerilliant or equivalent)
- m. Dimethyl dinitrobutane (DMDNB) (Acros or equivalent)

- n. 4-Nitrotoluene (4-NT) (Acros or equivalent)
- o. Tetryl, 1000 µg/mL (Cerilliant or equivalent)
- p. Autosampler vials - 2 mL GC vials, crimp or screw top, with or without 100-500 µL inserts (Agilent or equivalent)
- q. Injection port liners - 4 mm split-splitless, tapered, with or without glass wool (Agilent or equivalent)
- r. Injection port septa - standard low-bleed 11 mm (Agilent or equivalent)
- s. Autosampler syringes - Hamilton 701ASN 10 µL (or equivalent)
- t. Volumetric flask
- u. Acetone, Reagent grade

4 Standards and Controls

4.1 GC/ECD Testmix

The Testmix is a 10 µg/mL solution of EGDN, DMDNB, 4-NT, NG, 2,4-DNT, TNT, PETN, RDX, Tetryl, and HMX in acetone. Individual 100 µg/mL or 1000 µg/mL standard solutions of each component are purchased from Cerilliant, or equivalent. DMDNB and 4-NT are purchased as solids.

To prepare: A 100 µg/mL intermediate stock solution is prepared by adding 2 mL of each 1000 µg/mL liquid component and 2 mg of each solid component to a 20- mL volumetric flask, and diluting to volume with acetone.

The 10 µg/mL testmix is prepared by adding 1 mL of the 100 µg/mL stock solution to a 10-mL volumetric flask and diluting to volume with acetone. The testmix and intermediate stock solutions will be maintained in colored or amber vials in a refrigerator and have a shelf-life of two years. Record stock solution preparations in the Reagent Log. The Testmix is used to assess daily operating performance and continued integrity of the system.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Check to ensure that the GC wash vials are filled, the waste vials are empty, and all are in the appropriate positions.
- b. Record the remaining disk space on the hard drive. Use the Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- c. Record the line pressure of the building helium supply (carrier gas). The regulator should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the helium is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.
- d. Perform an analysis of the Testmix. Open the appropriate Testmix instrument method, and verify the parameters as listed in the 'Instrumental Conditions' section of this protocol. Set up a sequence, load the autosampler with a vial containing the Testmix, and start the analysis. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the chromatogram.
- e. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.2 As Needed Checks

The following steps are to be performed as needed based on system performance. Indicate completion in the appropriate QA/QC log.

- a. Replace the septum in the GC injection port.
- b. Replace the liner within the GC injection port.
- c. Check the GC syringe in the autosampler. Replace if needed.

7 Instrumental Conditions

Oven

Initial Temp: 50°C
Initial Time: 1.5 min
Ramp: 25°C/min
Final Temp: 250°C
Hold Time: 0.5 min
Equilibration Time: 1.0 min

Inlet/Injector

Inj Vol: 1.0 µL
Inlet temperature: 225°C
Injection mode: Split
Carrier gas: Helium, 99.99% (split)
Carrier mode: Constant flow
Pressure: 9.5 psi
Split ratio: 5:1

Column

Type: DB-5 MS
Length: 6 m (approximate)
Diameter: 0.25 mm
Film Thickness: 0.25 µm

Detector

Temperature: 275°C
Mode: Constant makeup flow
Makeup flow: 25 mL/min
Makeup Gas: Nitrogen

8 Decision Criteria

Verify the results of the Testmix.

- a. In order for the instrument to be considered in good operating condition, the EGDN, DMDNB, 4-NT, NG, 2,4-DNT, TNT, PETN, RDX, and Tetryl should generate well resolved, Gaussian-shaped peaks with baseline separation.
- b. The retention times of the components should not deviate by $\pm 3\%$ compared to previous runs.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

12 Safety

The ECD uses a sealed Nickel 63 source that should not be opened or modified in any way. Radiation leak checks are performed approximately twice per year as a safety precaution. Only properly trained personnel will perform duties involved in the radiation leak testing of this instrument.

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Gas Chromatograph General Maintenance Protocol" (Inst 002) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	04/25/16	New document, previously existed in the Explosives Unit.
1	04/29/16	Section 3 fixed typo and concentration. Section 4 fixed typo. Added approximate to section 7. Section 8 added compound abbreviations to criteria.
2	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Added Section 4.1 for more detailed testmix information. Updated heading in Section 5. Added 'appropriate instrument support personnel' to Section 6.1 c & e. Updated 'Instrument Operation and Systems Support' in Section 13 and header.

Approval

Redacted - Signatures on File

Explosives (Chemistry)
Technical Leader: —

Date: 09/28/2018

IOSS Manager: —

Date: 09/28/2018

Chemistry Unit Chief: —

Date: 09/28/2018

QA Approval

Quality Manager: —

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Ion Chromatography (IC) System

1 Scope

This document addresses the performance monitoring (QA/QC) of the Ion Chromatography (IC) system. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Explosives (chemistry) and Chemistry Unit general physical and chemical analysis.

2 Principle

The IC system is a high performance liquid chromatography (HPLC) pump and a conductivity detector. The instrument can be configured to analyze anions or cations. The resulting chromatogram is analyzed based on retention time (measured in minutes). When an analyte elutes from the column and passes the detector, it produces a change in conductivity which is recorded as a peak in the chromatogram.

This performance monitoring protocol is based upon the manufacturer's recommendations. The definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Dionex ICS-2000 or ICS-2100 HPLC pump and suppressed conductivity detector, Dionex AS programmable autosampler, and Chromeleon Software (or equivalent)
- b. Instrumentation - Waters e2695 Separations module, Waters 432 Conductivity Detector, and Empower Software (or equivalent)
- c. Columns:
 - IonPac CS12A Analytical Column (Dionex or equivalent)
 - IC-Pak C M/D Analytical Column (Waters or equivalent)
 - IonPac AS19 Analytical Column (Dionex or equivalent)
 - IonPac AS22 Analytical Column (Dionex or equivalent)
 - IonPac AG19 Guard Column (Dionex or equivalent)
 - IonPac AG22 Guard Column (Dionex or equivalent)
 - IonPac CG12A Guard Column (Dionex or equivalent)
- d. Nitric Acid (HNO_3) (Reagent Grade)
- e. Ethylenediaminetetraacetic Acid (EDTA) (Reagent Grade)

- f. Thermo Dionex EGC III KOH RFIC Eluent Generator (Potassium Hydroxide), or equivalent
- g. Thermo Dionex EGC III MSA RFIC Eluent Generator (Methanesulfonic Acid), or equivalent
- h. Thermo Dionex EGC 500 K₂CO₃ Eluent Generator (Potassium Carbonate), or equivalent
- i. Deionized Water, 18 MΩ·cm Milli-Q or equivalent
- j. Sodium, Ammonium, Potassium, Chloride, Nitrite, Chlorate, Nitrate, Sulfate, Thiocyanate, Perchlorate, Calcium and Magnesium Standards for IC (1000 mg/L) (Fluka or equivalent)
- k. Syringe - 250 µL (Dionex or equivalent)

4 Standards and Controls

4.1 Anions Testmix

The Testmix is used to assess daily operating performance and continued integrity of the system.
To prepare:

Pipette 5 mL of each anion standard for IC (chloride, nitrite, chlorate, nitrate, sulfate, thiocyanate, and perchlorate) into a 250 mL volumetric flask, and dilute to the mark with deionized 18.2 MΩ·cm water. Shelf life is two years when stored refrigerated in a plastic bottle. This preparation may be appropriately scaled.

4.2 Cations Testmix

The Testmix is used to assess daily operating performance and continued integrity of the system.

To prepare:

Pipette 5 mL of each cation standard for IC (ammonium, potassium, sodium, calcium and magnesium) into a 250 mL volumetric flask, and dilute to the mark with deionized 18.2 MΩ·cm water. Shelf life is two years when stored refrigerated in a plastic bottle. This preparation may be appropriately scaled.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 100 MB of free space. Do not use if less than 100 MB remain.
- b. Check the level of deionized water in the reservoir and make sure there is sufficient volume to complete the sequence.
- c. Check the level of the waste container. Empty if necessary.
- d. For the Waters Cations system, set an appropriate base and sensitivity range.
- e. Perform an analysis of the appropriate Testmix. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the chromatogram.
- f. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.2 As Needed Checks

- a. Fill the needle wash reservoir (Waters Only).
- b. Replace the Eluent Generator.
- c. Replace the guard column.
- d. Replace the analytical column.

7 Instrumental Conditions

7.1 Dionex Cations

Mobile Phase:	Methanesulfonic acid (20mM), supplied from Eluent Generator
Pump Mode:	Isocratic
Flow Rate:	1.0 mL/min
Column:	Dionex IonPac CS12A 4x250mm with IonPac CG12A Guard 4x50mm
Column Temperature:	30°C

Inj Volume: 25 µL
Acquire Time: 15 minute minimum

7.2 Waters Cations

Mobile Phase: 3.0 mM Nitric Acid (HNO₃) / 0.1 mM EDTA
Pump Mode: Isocratic
Flow Rate: 1.0 mL/min
Column: Waters IC-Pak Cation M/D 3.9x150mm
Column Temperature: Ambient
Inj Volume: 10 µL
Acquire Time: 15 minute minimum

7.3 Dionex Anions (Potassium Hydroxide Method)

Mobile Phase: Potassium Hydroxide (gradient 20-80 mM)
Pump Mode: Multi-step gradient (20 mM at 0 min, 20 mM at 2 min, 30 mM at 9 min, 80 mM at 13 min, 80 mM at 21min, 20 mM at 21.1 min, 20 mM at 25 min)), supplied from Eluent Generator
Flow Rate: 1.0 mL/min
Column: Dionex IonPac AS19 4x250mm with IonPac AG19 Guard 4x50mm
Column Temperature: 30°C
Inj Volume: 25 µL
Acquire Time: 25 minute minimum

7.4 Dionex Anions (Potassium Carbonate Method)

Mobile Phase: Potassium Carbonate (10mM), supplied from Eluent Generator
Pump Mode: Isocratic
Flow Rate: 1.5 mL/min
Column: Dionex IonPac AS22 4x250mm with IonPac AG22 Guard 4x50mm
Column Temperature: 30°C
Inj Volume: 25 µL
Acquire Time: 16 minute minimum

8 Decision Criteria

Verify the results of the Testmix.

- a. In order for the instrument to be considered in good operating condition, all components from the appropriate Testmix should generate well-resolved, Gaussian-shaped peaks with baseline separation.
- b. A SNR of 3:1 will be the minimum response necessary to consider a response a peak.

- c. The retention times of the appropriate Testmix components should not deviate by $\pm 5\%$ compared to previous runs of the appropriate Testmix.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	04/25/16	New document, previously existed in the Explosives Unit.
1	04/29/16	Added 'or equivalent' to section 3 for the eluent generators. Section 6 removed the detector settings for the Dionex system.
2	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Added calcium and magnesium to Sections 3 j and 4.2. Updated heading in Section 5. Added 'appropriate instrument personnel' to Section 6.1 f. Changed from 10 min to 15 min in Sections 7.1 and 7.2. Updated 'Instrument Operation and Systems Support' in Section 13 and header.

Redacted - Signatures on File

Approval

Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Explosives (Chemistry
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Thermo Nicolet FTIRs

1 Scope

This document addresses the performance monitoring (QA/QC) of the Thermo Nicolet Fourier Transform Infrared (FTIR) bench spectrometer and various accessories. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, paint, explosives (chemistry), fire debris, and Chemistry Unit general physical and chemical analysis.

2 Principle

The Thermo Nicolet FTIR is a bench spectrometer that can be used in transmission mode or in conjunction with an accessory. The accessories include a Continuum microscope, a DuraSamplIR ATR (Attenuated Total Reflectance), a Golden Gate ATR, a Smart Orbit ATR, and an integrated iS50 ATR. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Thermo Nicolet 6700 Nexus or iS50 FTIR, and Omnic Software (or equivalent)
- b. Microscope Accessories: Thermo Nicolet Continuum (or equivalent)
- c. ATR Accessories: SensIR DuraSamplIR, Golden Gate, Smart Orbit, integrated iS50 (or equivalent)
- d. Compressed nitrogen to purge instrument and microscope, if appropriate
- e. Liquid nitrogen (for microscopes)
- f. Dewar Flask (for microscopes) (supplied with the instrument by Thermo Nicolet)
- g. Polystyrene Standards - 1.5 mil and 3.0 matte-finish films mounted on a card, or mounted in a metal frame, or as internal standards within the bench (Thermo Nicolet or equivalent)
- h. Slide containing a metal disk with a 100 micron pinhole, an open hole approximately 11 mm in diameter, and a 14 mm diameter gold mirror (for microscopes) (Thermo Nicolet or equivalent)

4 Standards and Controls

4.1 Performance Standards

The polystyrene films are used to assess operating performance, wavenumber assignment, and continued integrity of the system. The polystyrene standards used for this procedure require no preparation and do not expire. It is recommended by Thermo Nicolet that they are replaced if showing signs of wear or if results have drifted.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Choose the appropriate bench and/or accessory. If using an ATR accessory, insert it into the bench (if applicable). If using a microscope accessory, cool the detector with liquid nitrogen.
- b. Load a method that is appropriate for the bench/accessory and mode of detection (ATR or %T) being used. Verify all parameters using the 'Instrumental Conditions' section of this protocol.
- c. Collect an ambient background spectrum and sample spectrum of the polystyrene standard.
- d. Use 'Find Peaks' to label the major peak apexes. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the spectrum.
- e. Save the spectrum to the QA/QC polystyrene standards folder.
- f. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, align the bench/accessory and re-analyze the polystyrene. If the results are still poor or failing, contact appropriate instrument support personnel.

6.2 As Needed Checks

The following steps are to be performed as needed based on system performance, and can be performed more frequently if desired. If a problem is indicated by the failure of the 'Daily Checks', these steps can help to identify the cause of an instrument error. Indicate completion in the appropriate QA/QC log.

6.2.1 Interferogram Signal Evaluation

- a. Select the method (mode of detection) 'Bench %T' or equivalent.
- b. Clear the sample compartment of any material which would impede the IR beam.
- c. Monitor the interferogram signal under a gain setting of one (1.0).
- d. Record the peak-to-peak voltage of the interferogram (which is the sum of the absolute minimum and maximum peak values) on the QA/QC Log. This value reflects the amount of signal (in terms of voltage) being detected.
- e. If the signal value has dropped significantly, the beamsplitter can be automatically adjusted to improve the beam voltage throughput. Refer to the manufacturer's instrument manuals for further instructions or contact appropriate instrument support personnel.

6.2.2 Bench Evaluation

- a. Initialize the appropriate system validation/qualification program (ex. Val-Q/ValPro) from within Omnic.
- b. Open the appropriate bench.csv file (if applicable).
- c. Start the validation. The Nexus 6700 and iS50 will internally supply the polystyrene standards.
- d. Evaluate the validation report. It will specify if all tests pass or if any fail. If any fail, align the bench and repeat. When the results are acceptable, print the report. Printing the spectral data is optional.
- e. Save the spreadsheet data to the hard drive
- f. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be performed.

7 Instrumental Conditions

7.1 ATR Accessory

Mode:	Reflectance
Number of scans:	32
Resolution:	4
Scan range:	minimum 400 – maximum 4000 cm^{-1}

7.2 Microscope Accessory

Mode:	Transmission
Number of scans:	128
Resolution:	4
Scan range:	650-4000 cm^{-1} for MCT/A 475-4000 cm^{-1} for MCT/B
Objective and stage compensators:	0

8 Decision Criteria

8.1 Polystyrene

The Polystyrene spectrum is acceptable if the following four peaks are within a $\pm 4 \text{ cm}^{-1}$ window of the expected wavenumber. If values lie outside the specified range, align the bench/accessory and re-analyze the polystyrene. If the results are still poor or failing, contact appropriate instrument support personnel. The following values have been provided by Nicolet:

<u>Expected</u>	<u>Acceptable Range</u>
3025 cm^{-1}	3021 to 3029 cm^{-1}
1601 cm^{-1}	1597 to 1605 cm^{-1}
1028 cm^{-1}	1024 to 1032 cm^{-1}
906 cm^{-1}	902 to 910 cm^{-1}

8.2 Validation Report

The Validation Report generated will indicate whether the obtained values lie within the ranges specified by the manufacturer and provide a 'pass' or 'fail' result. All tests should pass.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
2	04/25/16	Updated instrumentation in sections 2 and 3 to remove model 560 and 670, and add the iS50 and integrated iS50 ATR. Changed Scan range in section 7.1 to allow for wider variable range.
3	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Updated heading in Section 5. Removed labeling requirement in Section 6.1. Added 'appropriate instrument support personnel' to Sections 6.2.1 e, 6.2.2 f, and 8.1. Updated 'Instrument Operation and Systems Support' in Section 13 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Toxicology
Technical Leader:

Date: 09/28/2018

Paints and Polymers
Technical Leader:

Date: 09/28/2018

Fire Debris Technical
Leader:

09/28/2018

Explosives (Chemistry)
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

ate: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the UV-Vis Spectrophotometer

1 Scope

This document addresses the performance monitoring (QA/QC) of the UV-Vis Spectrophotometer. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, and Chemistry Unit general physical and chemical analysis.

2 Principle

The UV-Vis Spectrophotometer is used to measure the absorbance of light in the ultraviolet and visible regions of the electromagnetic spectrum. All identifications of spectral regions are based on absorption band positions which are given in wavelength (nm). Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation – Thermo-Fisher Evolution 220 UV-Vis Spectrophotometer with Insight 2 software (or equivalent)
- b. 1.0 cm quartz cuvette cell (or equivalent)
- c. Caffeine (USP grade or equivalent)
- d. Analytical Balance (Mettler or equivalent)
- e. 1000 mL Volumetric Flask
- f. Deionized Water, 18.2 MΩ·cm (Milli-Q or equivalent)

4 Standards and Controls

4.1 Testmix

The Testmix is used to assess daily operating performance and continued integrity of the system. The Testmix for the UV-Vis is a 10 ppm standard solution of caffeine in water. To prepare, weigh 10 mg caffeine. Dilute to 1000 mL with deionized water in a volumetric flask. Label the solution with the preparation date, initials, and expiration date. Store the solution at room temperature. It has a shelf-life of 5 years.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log to indicate completion.

- a. Allow the spectrophotometer to warm up for at least 20 minutes.
- b. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- c. Perform an analysis of the Testmix. Verify use of the parameters as listed in the 'Instrumental Conditions' section of this protocol. Fill a cuvette with the Testmix. Start the analysis.
- d. Use 'Find Peaks and Valleys' to label max and min. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the absorbance spectrum of the Testmix with the y-axis scale ranging from 0-1 Absorbance Units.
- e. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

7 Instrumental Conditions

7.1 UV-Vis Spectrophotometer

Start: 360 nm
End: 220 nm
Data Interval: 0.5 nm
Scan Speed: 120.00 nm/min
Band Width: 1.0000 nm

8 Decision Criteria

8.1 Testmix

Compare the daily Testmix to a previous analysis. The spectrum should be consistent with the previous analysis.

The absorbance spectrum is acceptable if the following are within a ± 1 window of the expected Absorbance unit.

- Max = 272 nm
- Min = 245 nm

8.2 Performance Verification Standards

All tests performed during the performance verification must pass. If they do not, contact appropriate instrument support personnel.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems*

Support SOP Manual.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	06/21/06	New document which replaces original titled "Performance Monitoring Protocol (QA/QC) for the Perkin Elmer Lambda 40 UV-VIS Spectrophotometer."
1	09/21/09	Removed model number from sections 1 and 2. Added explanation of UV-Vis in section 2. Updated model number in section 3a. Added statement about documentation in section 4.1. Clarified performance verification in section 4.2. Added reference to 'Instrumental Conditions' in section 7.1c. Added section 7.1d for labeling and reference to 'Decision Criteria.' Changed wording of statement in section 7.2f to be consistent with other SOPs. Updated section 7.2 to indicate 'as needed.' Updated sections 7.2c, d and f to reflect terminology used in new software. Updated conditions in section 8.1 based on new instrument methodology. Clarified section 9.1 with more specific decision criteria.
2	10/04/18	Removed Perkin Elmer from the title. Updated Section 1 Scope to include applicable disciplines/categories of testing. Removed Perkin Elmer from Section 2. Changed Perkin Elmer to Thermo-Fisher and added new instrument model to Section 3 a. Updated grade of caffeine in Section 3 c. Removed Section 3 b. Removed Section 4.2. Deleted Calibration section and updated heading in Section 5. Added "and Valleys" to Section 7.1 d. Removed Section 7.2. Removed Ordinate Mode and changed slit width to band width in Section 8.1. Added 'appropriate instrument support personnel' to Sections 7.1 e and 9.2. Updated 'Instrument Operation and Systems Support' in Section 14 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Toxicology
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

Redacted - Signatures on File

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Raman Spectrometers

1 Scope

This document addresses the performance monitoring (QA/QC) of the Raman Spectrometers (sample compartment and/or microscope). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, paint, explosives (chemistry), and Chemistry Unit general physical and chemical analysis.

2 Principle

A Raman spectrometer can be used to analyze samples in larger quantities in the sample compartment, if available, or in smaller quantities on the microscope, utilizing one or more objectives (e.g., 10X, 50X, 100X). In general, the signal for opaque samples can be maximized with a high numerical objective utilizing a microscope, while the signal for transparent samples can be maximized using a macro lens (i.e., sample compartment) or small magnification objective. The Raman spectrometer, either Fourier transform or dispersive, may utilize one or more excitation lasers (e.g., 1064 nm, 785 nm, 780 nm, 532 nm). In general, the signal will be more intense with a shorter wavelength excitation source; however, there is a trade off as samples may fluoresce and/or overheat with higher energy. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Thermo Almega XR Dispersive Raman Spectrometer System with sample compartment and microscope, Omnic Software (or equivalent)
- b. Thermo DXR Dispersive Raman Spectrometer System with Omnic Software (or equivalent)
- c. Thermo NXR FT-Raman Module with Omnic Software (or equivalent)
- d. Horiba Xplora Raman Microscope with LabSpec6 Software (or equivalent) and Bio-Rad Know-It-All (Horiba Edition) Software
- e. Polystyrene slide, rod, or disk, such as FT-Raman standard (Thermo or equivalent)
- f. Silicon and Polystyrene Slide (Horiba or equivalent)
- g. Potassium Bromide (KBr) Raman Test Standard (Thermo or equivalent)
- h. Alignment tool containing calibration slide with pinhole and white light (Thermo or

equivalent)

4 Standards and Controls

4.1 Performance Verification Standard

Polystyrene is used to assess daily operating performance and continued integrity of the Thermo Raman systems. Polystyrene requires no preparation and does not expire.

Silicon is used to assess daily operating performance and continued integrity of the Horiba Raman systems. Silicon requires no preparation and does not expire.

4.2 Alignment Tool (Thermo Only)

The alignment tool is used as needed to verify that the sample compartment and microscope are aligned and functioning properly. There is no sample preparation involved. The tool does not expire.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks

6.1.1 Microscope (Thermo Almega/DXR Only)

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Start Omnic and turn on the desired laser (if applicable) under 'Experiment Setup.' Allow time for the laser(s) to warm up and for the detector/ccd to cool. If the detector/ccd is not cool, a message will be displayed when data collection is attempted. The current temperature readout can also be viewed under the Advanced Tab in Experiment Setup. When the detector/ccd temperature setpoint has been reached, data collection can begin.
- b. Turn the microscope illuminator on.
- c. Set the operating parameters as listed in the 'Instrumental Conditions' section of this protocol.

- d. Place the polystyrene slide on the stage and focus. Ensure that the illuminator is off, then collect the sample spectrum. Perform a peak analysis by using the 'Find Peaks' option under the 'Analyze' menu. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the labeled spectrum.
- e. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.1.2 Sample Compartment (Thermo Almega Only)

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Start Omnic and turn on the desired laser (if applicable) under 'Experiment Setup'. Allow time for the laser(s) to warm up and for the detector/ccd to cool. If the detector/ccd is not cool, a message will be displayed when data collection is attempted. The current temperature readout can also be viewed under the Advanced Tab in Experiment Setup. When the detector/ccd temperature setpoint has been reached, data collection can begin.
- b. Set Operating Parameters as listed in the 'Instrumental Conditions' section of this protocol.
- c. Place the polystyrene rod in the sample compartment. Collect the sample spectrum. Perform a peak analysis by using the 'Find Peaks' option under the 'Analyze' menu. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the labeled spectrum.
- d. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.1.3 Sample Compartment (Thermo NXR Module Only)

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Replace the standard KBr beamsplitter with the CaF₂ beamsplitter. Start Omnic and verify that the 'Use Raman Accessory' is checked. Prepare to collect the Reference spectrum by setting the Laser to OFF, and the White Light to either Medium or Low, to coincide with sample analysis parameters. Set to collect for 256 scans (in the Experiment Setup/Collect Tab). Place the NMR tube holder containing the KBr standard in the sample compartment. Adjust the focus, laser power, and/or aperture to maximize the signal within the acceptable range (0-9). Close Experiment Setup, and select Collect Reference (under the Collect tab).

- b. Set Operating Parameters as listed in the 'Instrumental Conditions' section of this protocol.
- c. Analyze the polystyrene standard. Place the polystyrene in the sample compartment and focus in the Experiment Setup/Bench Tab by using the focus arrows to maximize the signal within the acceptable range (0-9). Collect the sample spectrum. Perform a peak analysis by using the 'Find Peaks' option under the 'Analyze' menu. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the labeled spectrum.
- d. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.1.4 Microscope (Horiba Only)

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Start the LabSpec6 software and verify the lasers are on.
- b. Place the Silicon sample on the stage and focus using the short working distance 100x objective. Perform the 'Full Autocalibration' procedure, and verify a 'Pass' result for each laser and grating combination.
- c. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.2 As Needed Checks (Thermo Only)

The following procedure will be performed as needed based on performance. Enter the appropriate information in the QA/QC log to indicate completion.

- a. Alignment of the Spectrometer:
Perform the appropriate alignment for the instrument in use. See appropriate instrument personnel for assistance if needed.
- b. System tuning (Almega and DXR Only):
Focus the alignment tool on the white light. Verify calibration by choosing 'Calibrate Instrument' under the 'Collect' pulldown and, if applicable, check the boxes for:
 - Laser frequency calibration
 - Wavelength calibration
- c. If all requirements are within specification or a successful result is returned, prepare

the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

7 Instrumental Conditions

7.1 Microscope (Thermo Almega/DXR Only)

Collect Tab

Set exposure time = 20 seconds
Set number of exposures = 2
Set final format - shifted spectrum (cm^{-1})
Cosmic ray rejection, to coincide with analysis set-up
Set correction to white light, if applicable
Set number of background exposures = 2

Bench Tab

Select microscope as 'Beam path/Accessory'
Select laser, to coincide with analysis set-up
Set laser power level to 100%
Select aperture, to coincide with analysis set-up
Select objective, to coincide with analysis set-up
Set resolution to same conditions as sample analysis
Set Grating Positions = multiple
Set maximum range limit to 3300 cm^{-1} , and minimum range limit to 200 cm^{-1}

7.2 Sample Compartment (Thermo Almega Only)

Collect Tab

Set exposure time = 20 seconds
Set number of exposures = 2
Set final format - shifted spectrum (cm^{-1})
Check 'Cosmic ray rejection'
Set correction to white light
Set number of background exposures = 2

Bench Tab

Select '180-degree' as 'Beam path/Accessory'
Select laser, to coincide with analysis set-up
Set Focus, 'Side to side', and 'Up/down' parameters to achieve maximum signal
Set laser power level to 100%
Select aperture, to coincide with analysis set-up
Set resolution to same conditions as sample analysis
Set Grating Positions = multiple
Set maximum range limit to 3300 cm^{-1} , and minimum range limit to 200 cm^{-1}

7.3 Sample Compartment (Thermo NXR Only)

Set laser power level to coincide with analysis set-up
Set Focus, 'Side to side', and 'Up/down' parameters to achieve maximum signal
Set final format - corrected spectrum (cm^{-1})
Set cosmic ray rejection, to coincide with analysis set-up
Set white light to OFF
Set maximum range limit to 3701 cm^{-1} , and minimum range limit to 100 cm^{-1}
Set number of scans = 64
Detector = InGaAs
Beamsplitter = CaF_2
Optical Velocity = 0.3165

7.4 Microscope (Horiba Only)

Autocalibration parameters set by the system.

8 Decision Criteria

- The Polystyrene spectrum is acceptable if all peaks are within $\pm 5 \text{ cm}^{-1}$ of the expected values, listed below (in cm^{-1}):

621 796 1001 1032 1155 1451 1583 1602 2852 2905 3054
- The Autocalibration for the Horiba Raman system should result in a Pass result for all lasers and gratings.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

Almega Users Guide.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	11/12/14	New document to replace "Performance Monitoring Protocol (QA/QC) for the Thermo Almega Raman Spectrometer" (Inst 503) <i>Instrument Operation and Support Subunit SOP Manual</i> .
1	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Updated heading in Section 5. Added 'appropriate instrument support personnel' to Sections 6.1.1 e, 6.1.2 a & d, 6.1.3 d, 6.1.4 c and 6.2 c. Added illuminator to Section 6.1.1 d. Updated 'Instrument Operation and Systems Support' in Section 13 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Paints and Polymers
Technical Leader:

Date: 09/28/2018

Explosives (Chemistry)
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Minolta CR-241 Chroma Meter

1 Scope

This document addresses the performance monitoring (QA/QC) of the Minolta CR-241 Chroma Meter. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Paint and Chemistry Unit general physical and chemical analysis.

2 Principle

The Minolta CR-241 Chroma Meter, herein referred to as the colorimeter, is a device used for colorimetry measurement. Colorimetry provides a tristimulus color measurement of surfaces (e.g., automotive finishes). Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Minolta CR-241 Chroma Meter (or equivalent)
- b. Calibration Standard - White Calibration Plate (Currently Serial Number 11876001) (Konica Minolta or equivalent)

4 Standards and Controls

4.1 White Calibration Plate

The white calibration plate is provided by Konica Minolta and stored with the instrument. No preparation is required. It does not expire.

5 Calibration

The colorimeter must be calibrated prior to each use and when the measurement selector is changed.

- a. With the instrument on and ready for use, select the desired measurement area based on the sample size.
- b. Press the CALIBRATE button on the upper left side of the key pad. This will retrieve the previous settings, which will be shown in the display located in the upper right

corner of the base.

- c. Ensure that the values are set in Yxy color space. If they are not, press the COLOR SPACE SELECT button until the proper setting is displayed.
- d. Place the white calibration plate on the specimen stage and focus the instrument until the dots on the plate and the white surface appear sharp through the viewfinder.
- e. Adjust the specimen stage so that the measurement area is centered between the two dots on the calibration plate.
- f. Press the MEASURE button. A series of three measurements will be taken. Once calibration is complete, the new values will appear in the display.
- g. Evaluate the results based on the 'Decision Criteria' section of this protocol.
- h. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Daily Checks

The colorimeter must be calibrated prior to each use and when the measurement selector is changed. Refer to the 'Calibration' section of this protocol.

7.2 Operation

For analysis, samples must have a minimum area of 0.3 mm and should be relatively flat. If a sample is greater than or equal to 1.8 mm in diameter, adjust the measurement area selector accordingly.

- a. Place the sample on the specimen tray and focus the instrument on the sample.
- b. After focusing, choose the appropriate color space for analysis. This is accomplished by depressing the COLOR SPACE SELECT key (located in the upper right corner of the scope base). For a complete list of color spaces, please refer to the Operator's manual (pg. 13). Note: Munsell is used for automotive finishes.

- c. Press the MEASURE button.
- d. The instrument will measure the sample in the desired color space and display the results on the digital screen (located just above the COLOR SPACE SELECT key). If desired, a hard copy can be printed by depressing the DISPLAY PRINT key (located just below the COLOR SPACE SELECT key).
- e. When analysis is complete, turn the instrument off.

8 Instrumental Conditions

8.1 Chroma Meter

Measurement area: 1.8 or 0.3 mm (diameter)
Mode: Calibrate
Color space: Yxy

9 Decision Criteria

Compare the displayed values with the values printed on the inside cover of the calibration plate. Currently, calibration plate serial number 11876001 is in use and the values are:

C	Y 93.1	x 0.3126	y 0.3190
D ₆₅	Y 93.1	x 0.3151	y 0.3318

If the values are in agreement, the instrument is now ready for the analysis of samples. If the values do not match, enter the standard (actual) values for the white calibration plate to correct the calibration. Record the correction, operator's name, date, and comments in the QA/QC log.

10 Calculations

Not applicable.

11 Measurement Uncertainty

Not applicable.

12 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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.

14 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	06/21/06	New document which replaces original titled "Performance Monitoring Protocol (QA/QC) for the Minolta CR-241 Chroma Meter."
1	03/08/12	Changes made to equipment and instrument vendor name in Sections 3d and 4.1. Removed minor deviation statement from Section 8. Adjusted Decision Criteria in Section 9 to align with requirements of new calibration plate.
2	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Added 'appropriate instrument support personnel' to Section 5 h. Updated heading in Section 6. Updated 'Instrument Operation and Systems Support' in Section 14 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Paints and Polymers
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Dynex DSX Immunoassay System

1 Scope

This document addresses the performance monitoring (QA/QC) of the Dynex DSX Immunoassay System. This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Toxicology.

2 Principle

The Dynex DSX Immunoassay System is an automated analyzer designed to perform all functions necessary for running enzyme-linked immunosorbent assays (ELISA). This includes dispensing standards, samples and reagents, as well as washing and reading plates. Automated procedures may be written using the software within the system, in order to follow the instructions of different commercially available kits.

All analyte detection is based upon a reaction that results in a color change. Absorbance measurements of a sample are taken after the reaction has been stopped by a stopping reagent. Calibrators are made up in house. These are analyte solutions in an appropriate biological matrix which contain either no analyte (negative calibrator), a moderate amount of analyte (the cutoff calibrator) or a large amount of analyte (high positive calibrator). Cutoff levels can be chosen by the user.

This performance monitoring protocol is based upon the manufacturer's recommendations. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation – Dynex DSX Immunoassay System - ELISA
- b. Isopropanol, reagent grade
- c. Deionized Water, 18 MΩ·cm Milli-Q or equivalent
- d. Materials and reagents as listed in appropriate Toxicology SOPs

4 Standards and Controls

Not applicable.

5 Sampling or Sample Selection

Not applicable.

6 Procedure

6.1 Daily

- a. Follow the directions in the appropriate Toxicology SOP for specific step-by-step instructions for navigating the DSX Revelation software.
- b. Evaluate the results based on the 'Decision Criteria' section of appropriate Toxicology SOP.
- c. Prepare the documentation as outlined in the "General Instrument Maintenance Protocol." if all requirements are within specification. If any requirements fail, contact appropriate instrument support personnel.

7 Instrumental Conditions

The instrumental conditions are set upon installation, and need not be changed.

8 Decision Criteria

8.1 Daily Checks

Evaluate the results using the Decision Criteria section of the corresponding analyte-specific Toxicology SOP.

8.2 Pass/Fail

If all requirements under 'Decision Criteria' are within specification, then the instrument may be used for casework for that day. If any fail, refer to the "General Instrument Maintenance Protocol" for the appropriate procedure to follow.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Not applicable.

12 Safety

Take standard precautions for the handling of all biologicals, chemicals, reagents, and standards. Some of the chemicals may be carcinogenic. Refer to the *FBI Laboratory Division 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.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

Toxicology SOP Manual.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	06/21/06	New document.
1	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Deleted Calibration section and updated heading in Section 5. Added 'appropriate instrument support personnel' to Section 7.1 c. Updated 'Instrument Operation and Systems Support' in Section 14 and header.

Redacted - Signatures on File

Approval

Toxicology Technical
Leader: -

Date: 09/28/2018

IOSS Manager: -

Date: 09/28/2018

Chemistry Unit Chief: -

Date: 09/28/2018

QA Approval

Quality Manager: -

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for Balances

1 Scope

This document addresses performance monitoring (QA/QC) of analytical balances in the Chemistry Unit (CU). It is applicable to CU personnel utilizing balances to make a significant measurement (i.e., a measurement that requires an estimation of measurement uncertainty).

2 Principle

A balance is an instrument used to measure mass to a high degree of precision and accuracy. All balances and the mass reference standards are calibrated annually by an external calibration service provider (see *FBI Laboratory Practices for the Calibration and Maintenance of Equipment*). Many of the balances listed below adjust automatically overnight, with a change in ambient conditions, or when prompted by a user. These adjustments are often referred to as an autocalibration, but are different from a true calibration and will be considered an adjustment. The calibration status of a balance will be checked prior to conducting a significant measurement (i.e., a measurement that requires an estimation of measurement uncertainty) to ensure that any adjustments to the balance have not affected the calibration status of the balance. Other definitions and guidelines for following this protocol are outlined in the *Instrument Operation & Systems Support- General Instrument Maintenance Protocol*.

3 Equipment/Materials/Reagents

3.1 Balances

Refer to Resource Manager in Forensic Advantage (FA) for a listing of balances.

4 Standards and Controls

4.1 Mass Reference Standards

Refer to Resource Manager in FA for a listing of mass reference standards.

5 Calibration

Applicable balances and mass reference standards (see Resource Manager in FA) will be calibrated annually by an external calibration service provider (see *FBI Laboratory Practices for the Calibration and Maintenance of Equipment*). Balances and mass reference standards will be clearly marked with the calibration date and calibration due date. Original calibration certificates and supporting records (if applicable) will be maintained by CU.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Calibration Check

Only select balances have been designated for use in significant measurements. All balances are clearly marked as 'traceable' or 'not traceable' to indicate whether or not they can be used for a significant measurement. The following 'Calibration Check' procedure will be performed on 'traceable' balances prior to the first use of the balance to make a significant measurement on a given day. Since the mass reference standards are sent out annually for calibration, the daily 'Calibration Check' will be waived during the time period when the weight sets are out for external calibration.

Non-significant measurements on balances (whether 'traceable' or 'not traceable' balances) do not require a 'Calibration Check'.

- a. Verify that the balance is clean.
- b. If available on the particular balance, run the internal 'autocalibration' feature.
- c. Verify that the balance is properly zeroed.
- d. Refer to the applicable log sheet and measure each of the indicated weights (also shown below in section 8, for example a 0.1 mg resolution balance requires the measurement of the 100 milligram, 1 gram, and 10 gram weights), ensuring that the balance is properly zeroed between measurements. Handle the weights carefully with the provided forceps. Allow the balance to stabilize before recording the observed weight.
- e. Refer to the 'Decision Criteria' on the log sheet (and shown below in section 8). If a measured weight is outside of the 'Tolerance' range, provide the data to the CU Quality Assurance Program Manager for assessment of the calibration status of the balance. Record the appropriate information on the log sheet.
- f. If a balance calibration is found to be out of specification, the IOSS Manager or appropriate instrument support personnel will determine the corrective action to be taken.

8 Decision Criteria

Compare the measured weight with the tolerances listed in the table below. Each balance log sheet is labeled with the balance resolution and the corresponding tolerances.

Balance Resolution	'Calibration Check' Weights	Tolerance
0.1 mg (e.g., Mettler ML104-1)	100 mg (Rice Lake T549)	± 0.3 mg
	1 g (Rice Lake T549)	± 0.3 mg
	10 g (Rice Lake T549)	± 0.3 mg
0.001 g / 0.01 g (e.g., PJ-360-1)	2 g (Rice Lake T549)	± 0.002 g
	20 g (Rice Lake T549)	± 0.002 g
	100 g (Rice Lake T549)	± 0.02 g
0.1 g (e.g., Denver SI-8001-2)	5 g (Rice Lake T549)	± 0.2 g
	500 g (Troemner 1000045859)	± 0.2 g
	2 kg (Troemner 1000045858)	± 0.2 g

9 Calculations

Not applicable.

10 Measurement Uncertainty

Measurement uncertainty information for CU balances is maintained in a binder and electronically (e.g., Excel® spreadsheets) by the CU Quality Assurance Program Manager. The Type A repeatability uncertainty component of the balances will be updated annually using the 'Calibration Check' data.

Measurement uncertainty worksheets are available on Chemnet. The expanded uncertainty is provided at a 99.7% confidence level on the worksheets. If a weight is determined by the difference of more than one weighing (i.e., weight by difference) then the expanded uncertainty for the measurement will be calculated as the root-sum-square (RSS) of the expanded uncertainty of each weighing.

11 Limitations

Only properly trained personnel will perform duties involved in the maintenance or troubleshooting of these instruments. As mentioned in section 7, forceps should always be used

when handling weights.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. 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.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*

"Chemistry Unit Procedures for Estimating Measurement Uncertainty" (CUQA 13) *Chemistry Unit Quality Assurance and Operations Manual*

ASCLD/LAB-International Policy on Measurement Traceability

ASCLD/LAB-International Policy on Measurement Uncertainty

FBI Laboratory Operations Manual

FBI Laboratory Safety Manual

Rev. #	Issue Date	History
2	01/23/17	Removed 'external calibration requirements' language from sections 2 and 5. Updated balance models in section 3. Updated storage locations of weights in section 4.1. Changed documentation to records, and QATU to FASU in section 5. Specified that CU maintains copies of calibration certificates in section 5. Updated tables in section 8 (removed '0.01 g' resolution table, changed balance models in table where applicable). Changed Type A data recalculation frequency to annually in section 10. Added reference.
3	10/04/18	Revised section 1 to include personnel. Minor grammar revision to section 2 and updated IOSS name (also updated IOSS name in sections 7 and 13). Revised sections 3, 4, and 5 to remove specific balances/mass reference standards; added section 3.1 and moved mass reference standards to section 4.1. Edited section 5 to reflect original calibration records are maintained in CU. Updated heading in Section 6. Minor edit to section 7.1 for brevity and included sentence addressing non-significant measurements. Removed reference to gloves since weights are to be handled with forceps. Minor edits to table in section 8 for clarity.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader:

Date: 09/28/2018

Toxicology
Technical Leader:

Date: 09/28/2018

Metallurgy
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for Micrometers and Calipers

1 Scope

This document addresses performance monitoring (QA/QC) of calipers and micrometers. This document applies to personnel using the associated instrument(s)/equipment in the following disciplines/categories of testing: Chemistry Unit general physical and chemical analysis.

2 Principle

Micrometers and calipers are instruments used to measure physical dimensions to a high degree of precision and accuracy. All micrometers, calipers and standard gauge blocks in the Chemistry Unit (CU) are calibrated once each year in accordance with Laboratory Operations Manual (LOM) and Quality Assurance Manual (QAM) requirements. The calibration status of a micrometer or caliper will be checked prior to conducting any significant measurement (i.e., a measurement that requires an estimation of measurement uncertainty). Other definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Numerous micrometers and calipers are available in the CU. Traceability has been established only for the following instruments: Mitutoyo Micrometers (Serial Nos. FBI-014 and 8042323) and Mitutoyo Calipers (Serial Nos. 0033307 and 7107481).
- b. Thickness Reference Standards- Grade B (MG Gauge Block Set-Serial No. 50462).

4 Standards and Controls

4.1 Thickness Reference Standards (Gauge Blocks)

The thickness reference standards are stored in a marked wooden container in room 4283.

5 Calibration

All micrometers, calipers and the thickness reference standard set (gauge blocks) will be calibrated annually by a certified and licensed service provider that meets the LOM and QAM requirements. Each micrometer, caliper and the thickness reference standard set is clearly marked with the calibration date and calibration due date. Supporting documentation and certificates are maintained in accordance with the LOM.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Calibration Check

While all of the micrometers and calipers in CU are calibrated annually, only select micrometers and calipers have been designated for use in significant measurements. All micrometers and calipers are clearly marked as 'traceable' or 'not traceable' to indicate whether or not they can be used for a significant measurement. The following 'Calibration Check' procedure will be performed on 'traceable' micrometers and calipers prior to the first use of the instrument to make a significant measurement on a given day. Since the thickness reference standards are sent out annually for calibration, the daily 'Calibration Check' will be waived during the time period when the thickness reference standard set is out for external calibration.

Non-significant measurements on calipers and micrometers (whether 'traceable' or 'not traceable' instruments) do not require a 'Calibration Check'.

- a. Verify that the jaws of the instrument are clean.
- b. Verify that the instrument is properly zeroed.
- c. Refer to the applicable log sheet and measure each of the indicated reference gauge blocks (also shown below in section 8), ensuring that the instrument is properly zeroed between measurements. Handle the gauge blocks carefully with lint-free gloves.
- d. Refer to the 'Decision Criteria' (shown below in section 8). If a measured dimension is outside of the 'Tolerance' range, remove the instrument from service and provide the data to the Metallurgy Technical Leader for assessment of its calibration status. Record the appropriate information on the log sheet.
- e. If a micrometer or caliper calibration is found to be out of specification, the Metallurgy Technical Leader, the IOSS Manager or appropriate instrument support personnel will determine whether the instrument will be repaired and recalibrated (by an external service provider), or retired/replaced.

8 Decision Criteria

Compare the measured dimension with the tolerances listed in the table below. Each instrument log sheet is labeled with the instrument resolution and the corresponding tolerances.

Instrument Resolution	'Calibration Check' Gauge Blocks	Tolerance
-----------------------	----------------------------------	-----------

.001 inches Mitutoyo Micrometers	.050 inches (MG Block #61083)	± 0.001 inches
	0.500 inches (MG Block #67135)	± 0.001 inches
	1.000 inches (MG Block #70755)	± 0.001 inches

.0005 inches Mitutoyo Calipers	.050 inches (MG Block # 61083)	± 0.001inches
	0.500 inches (MG Block #67135)	± 0.001 inches
	2.000 inches (MG Block #70927)	± 0.001 inches

9 Calculations

Not applicable.

10 Measurement Uncertainty

Measurement uncertainty information for CU micrometers and calipers is maintained in a binder and/or electronically in the Chemistry Unit. The 'Calibration Check' data will be collected at least every 6 months and used to update the Type A repeatability uncertainty component of these instruments.

Measurement uncertainty worksheets are available on Chemnet. The expanded uncertainty is provided at a 99.7% confidence level on the worksheets for a single measurement.

11 Limitations

Only properly trained personnel will perform duties involved in the maintenance or troubleshooting of these instruments.

12 Safety

As mentioned in section 7, lint-free gloves should always be used when handling the thickness reference standards. Take universal precautions when making examining bio-hazardous evidence. Personal protective equipment will be worn when the potential for a biohazard exists. Refer to the *FBI Laboratory Safety Manual* for detailed information on the proper handling of bio-hazardous materials.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*

"Chemistry Unit Procedures for Estimating Measurement Uncertainty" (CUQA 13) *Chemistry Unit Quality Assurance and Operations Manual*

FBI Laboratory Operations Manual

FBI Laboratory Safety Manual

Rev. #	Issue Date	History
0	02/05/14	New document.
1	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Changed reference to LOM and QAM in Sections 2 and 5. Updated heading in Section 6. Added 'appropriate instrument support personnel' to Section 7.1 e. Changed 'Metallurgy Subunit Manager' to 'Chemistry Unit' in Section 10. Updated 'Instrument Operation and Systems Support' in Section 13 and header.

Approval

Redacted - Signatures on File

Metallurgy
Technical Leader:

Date: 09/28/2018

Paints and Polymers
Technical Leader:

Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Scanning Electron Microscope (SEM) / Energy Dispersive X-ray Spectrometer (EDS)

1 Scope

This document addresses the performance monitoring (QA/QC) of the Scanning Electron Microscope (SEM) / Energy Dispersive X-ray Spectrometer (EDS). This document applies to personnel using the associated instrument(s)/equipment in the following disciplines/categories of testing: Paint, explosives (chemistry), drug chemistry, and Chemistry Unit general physical and chemical analysis.

2 Principle

The SEM/EDS is utilized primarily to characterize the elemental composition of a material. Because this instrumentation is dependent upon a determination of the energy of detected X-rays, it is necessary to ensure the instrument is performing optimally for the intended analysis.

SEM can be utilized for morphological and metrological determinations; therefore image quality and measurement accuracy will need to be determined for these examinations. For routine analysis the magnification accuracy is sufficient. When a measurement is required with reportable accuracy, a calibrated measurement standard will be employed. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Scanning Electron Microscope (SEM) – JEOL JSM 6510LV, JEOL JSM6610, TESCAN Vega 3 XMU (or equivalent)
- b. Energy Dispersive Spectrometer (EDS) - EDAX (or equivalent)
- c. Ruler, metric - Fisher Scientific (or equivalent)
- d. Energy Dispersive X-ray processing software – EDAX Genesis (or equivalent)
- e. Spectrum Library Identification and Classification Explorer (SLICE) software, xk, Inc (or equivalent)
- g. Manganese (Mn) standard, polished (or equivalent)
- h. Magnification standard - Geller MRS-3 SEM (or equivalent)

4 Standards and Controls

Prior to each use of the SEM/EDS, a determination of the ability to perform elemental identification will be made by confirming system energy calibration using the X-ray lines of the pure element standard manganese (Mn).

5 Calibration

Calibration of the EDS will only be performed if the X-ray lines are shifted from their expected positions in the spectrum of the pure element standard manganese (Mn) by more than 30 eV. This procedure will be performed by properly trained personnel.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Daily Checks

- a. Collect a spectrum from the pure element standard manganese (Mn).
- b. Recall verification spectrum of previous pure element.
- c. Compare spectra. Observe and compare peak shape, peak width, high to low energy peak ratio, shape of background, peak artifacts, and system peaks.
- d. If the peaks observed are shifted from their previously established positions by more than 30 eV, then contact appropriate instrument support personnel.
- e. Print spectrum, initial, date and insert into appropriate section of the QA/QC log.

7.2 Magnification Standardization

Magnification standardization will be performed, as follows, when a value will be reported. Additional steps may need to be taken in order to report a value (e.g., measurement uncertainty calculations, traceability considerations).

- a. Place the Geller MRS-3 SEM magnification standard into SEM chamber and evacuate.
- b. Set specimen tilt to 0 degrees (perpendicular to the electron beam).
- c. Adjust the instrumental conditions to be similar to those used to image the object

from which an accurate measurement is required.

- d. Bring the Geller MRS-3 SEM magnification standard into focus using stage Z.
- e. Take one digital image of an appropriate pattern on the Geller standard. Different pattern selections are available depending on the magnification selected. Examples are provided below:

<u>Pattern</u>	<u>Spacing</u>
50X	largest bar plus space = 0.5 mm
100X	largest bar plus space = 0.5 mm
1,000X	middle bar plus space = 50 μ m
10,000X	smallest bar plus space = 2 μ m

- f. Using a ruler, measure the features in the digital images and calculate the actual magnification.
- g. Calculate % magnification error, and apply the correction to the measurement from the structure of interest.
- h. Record results in QA/QC log.
- i. Once a magnification error has been determined for a specific set of analytical conditions, error correction can be applied to any subsequent measurements obtained under those conditions.

8 Instrumental Conditions

8.1 Imaging

Detector type (e.g. secondary or backscatter) and values for accelerating (high) voltage, working distance, spot size, beam intensity, stigmation, focus, brightness, and contrast are established at the operator's discretion based on image quality desired.

8.2 Magnification

Instrumental conditions will be the same as those required to image a material of interest from which a precise measurement is required.

8.3 EDS Detector

Detector response: Mn
Beam voltage: 25KV

Working distance and beam intensity/spot size will be set at the operator's discretion.

9 Calculations

magnification = (image dimension)/(object dimension)

% magnification error =

$[(\text{displayed magnification} - \text{measured magnification}) / \text{measured magnification}] \times 100$

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of the SEM and/or EDS.

12 Decision Criteria

- a. Detector response:
In order for the instrument to be considered in good operating condition, the manganese spectrum must appear generally similar to the previously collected manganese spectra. The spectrum should exhibit a similar high-to-low energy peak ratio, Gaussian peak shape, a minimum SNR of 3:1, and the absence of any significant spectral artifacts. Changes in the low-to-high peak intensity ratio may indicate accumulation of ice on the crystal face.
- b. Energy characterization:
If the measured peak centroid energy is more than 30eV from the theoretical average Mn K α peak energy of 5.895 keV, a detector calibration will be performed in accordance with the manufacturer's recommendations.
- c. If all requirements are within specification, prepare documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be performed.

13 Safety

General precautions common to electron microscope laboratories include: Venting of P-10 gas, venting or filtering of roughing pump discharge, and avoidance of direct exposure to beryllium metal. Under normal operator conditions the instrument poses no known hazards.

Use universal precautions when handling potentially biohazardous materials. Take standard

precautions for the handling of chemicals and sharp cutting instruments. Refer to the *FBI Laboratory Safety Manual* and appropriate SDSs for additional required practices and precautions.

14 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

Goldestein, Newbury, Echlin, Joy, Romig, Lyman, Fiori, Lifshin, *Scanning Electron Microscopy and X-ray Microanalysis*, Second Edition, Plenum Press, 1992.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
2	12/05/11	Removed dead time requirement from section 8.3.
3	10/04/18	Updated Section 1 Scope to include disciplines/categories of testing. Added JEOL JSM6610 to Section 3 a. Updated heading in Section 6. Removed specific software titles from Section 7.1 b. Removed requirement to overlay spectra in Section 7.1 e. Added 'appropriate instrument support personnel' to Sections 7.1 d and 12 c. Added additional steps for reporting to Section 7.2. Added detector types in Section 8.1. Added 'EDS' to Section 8.3 title. Added theoretical value in Section 12 b. Removed outdated cryogen hazard from Section 13. Updated 'Instrument Operation and Systems Support' in Section 14 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
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Date: 09/28/2018

Metallurgy
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Date: 09/28/2018

Paints and Polymers
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Explosives (Chemistry)
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Date: 09/28/2018

IOSS Manager:

Date: 09/28/2018

Chemistry Unit Chief:

Date: 09/28/2018

QA Approval

Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for ICP-MS

1 Scope

This document addresses the performance monitoring (QA/QC) of the Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). This document applies to personnel using the associated instrument(s)/equipment in the following disciplines/categories of testing: Toxicology, explosives (chemistry), and Chemistry Unit general physical and chemical analysis.

2 Principle

The ICP-MS system is comprised of an Inductively Coupled Plasma-Mass Spectrometer with a collision cell. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation – Thermo-Fisher ICAP Q with collision cell
- b. Plasma Gas - Argon, 99.996% (Arcet or equivalent)
- c. Helium – 99.9999% (Arcet or equivalent)
- d. Thermo Tune-B solution or equivalent (Thermo or equivalent)
- e. Thermo Setup solution or equivalent (Thermo or equivalent)
- f. 50 mL polypropylene test tubes

4 Standards and Controls

4.1 Tuning Solution

The Thermo Setup solution is used for tuning the mass spectrometer.

4.2 Performance Verification Standard

The Thermo Tune-B solution is analyzed daily to assess operating performance, mass assignment, and continued integrity of the system. The Thermo Tune-B solution will be evaluated prior to the analysis of evidence. This solution will also be used to tune the source of the ICP-MS.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

The following steps will be performed when the instrument will be in use. Enter the appropriate information in the QA/QC log to indicate completion. Refer to the manufacturer's instrument manuals as needed if any parts require cleaning or replacement.

- a. Check the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- b. Verify that the argon line has 80 p.s.i. or above.
- c. Check the torch to see if there is any visible residue or discoloration present. Clean the torch if it is dirty.
- d. Check the sampler cone for dirt/residue build up. Clean the cones if they are dirty.
- e. Check the sample pump tubing to verify that it is in good working condition (i.e., no flat spots or blockages present). Change the pump tubing as needed.
- f. Check the color of the pump oil.
- g. Perform an analysis of the Performance Verification Standard using Thermo Tune-B solution. Open the appropriate instrument protocol and start the analysis. The report from the daily performance check will appear on the screen. Print and evaluate the results based on the 'Decision Criteria' section of this procedure.
- h. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, check the condition of the peristaltic pump tubing, the condition of the cones, and the torch and injector position/cleanliness. Most performance problems arise from the condition of

the sample introduction system, torch, or cones. Significant changes in mass intensities, oxide ratios, doubly charged ion ratios, or increases in background may indicate the need to initiate further optimization procedures or maintenance procedures. Refer to the manufacturer's instrument manuals for details. If those measures are unsuccessful, contact appropriate instrument support personnel.

7 Instrumental Conditions

Conditions are preset in the system based on the tune.

8 Decision Criteria

8.1 Performance Verification Standard

Verify the results of the daily Performance Verification Standard

Background:	< 1 cps @ Mass 4.5
Background:	< 3 cps @ Mass 220
Li Sensitivity:	> 25,000 cps
Co Sensitivity:	> 50,000 cps
In Sensitivity:	> 110,000 cps
U Sensitivity:	> 150,000 cps
CeO/Ce:	≤ 0.03
Ba ²⁺ / Ba ⁺ :	≤ 0.03
Li, Co, In, and U Stability:	≤ 3%

8.2 Performance Verification Kinetic Energy Discrimination (KED)

Verify the results of the daily Performance Verification KED.

Background:	< 1 cps @ Mass 4.5
Background:	< 3 cps @ Mass 220
Co Sensitivity:	> 15,000 cps
In Sensitivity:	> 15,000 cps
U Sensitivity:	> 40,000 cps
Co/CIO:	> 18
CeO/Ce :	≤ 0.02
Li, Co, In, and U Stability:	≤ 3%

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

12 Safety

The current instrument model contains safety interlocks to prevent the torch box from being opened while the plasma is ignited. Do not attempt to defeat the interlock.

Take standard precautions for the handling of all chemicals, reagents, and standards. 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. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	03/18/15	New document that replaces a previous document entitled "Performance Monitoring Protocol (QA/QC) for the Perkin Elmer ICP-MS."
1	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Updated heading in Section 5. Added 'appropriate instrument support personnel' to Section 6 h. Added statement regarding instrument conditions in Section 7. Updated 'Instrument Operation and Systems Support' in Section 13 and header.

Approval

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Date: 09/28/2018

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Date: 09/28/2018

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Quality Manager:

Date: 09/28/2018

Performance Monitoring Protocol (QA/QC) for the Rigaku MiniFlex X-Ray Diffractometer (XRD)

1 Scope

This document addresses the performance monitoring (QA/QC) of the Rigaku MiniFlex X-ray diffractometer (XRD). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Explosives (chemistry), paint, drug chemistry, and Chemistry Unit general physical and chemical analysis.

2 Principle

The Rigaku MiniFlex XRD uses an X-ray source, a goniometer, and a detector to generate an X-ray diffraction pattern for the sample being analyzed. The generated pattern is compared to X-ray patterns in the library of the International Center for Diffraction (ICDD), the pattern of a known standard, or the pattern of other material of known origin.

This performance monitoring protocol is based upon the manufacturer's recommendations. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation – Rigaku MiniFlex II, MiniFlex 600, with PDXL Software (or equivalent)
- b. International Center for Diffraction Data (ICDD) Library
- c. NIST SRM 640c Silicon Powder (or equivalent traceable standard)

4 Standards and Controls

The silicon standard is used to ensure that the sample holder and X-ray tube are aligned and functioning properly. The standard is used to verify performance and continued integrity of the system. This standard does not expire.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- b. Place the silicon standard in the instrument.
- c. Open the 'General Measurement' or 'Standard Measurement' window, enter the appropriate information pertaining to the standard, and start the measurement.
- d. Using the PDXL software, open the silicon pattern just created by selecting 'Load' and the recently analyzed silicon standard data file.
- e. In the 'Profile View' tab, unclick the buttons for 'ResidualGraph' (optional) and 'Peakbar 1'. Click 'Create Report' and select "Silicon Standard QA/QC Printout" (or equivalent). Click 'Create Report' and resize the diffractogram so that all information fits on one page. Alternatively, print the diffraction pattern and peak list.
- f. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable print the report.
- g. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

7 Instrumental Conditions

The X-ray generator parameters, detector, and scan mode are fixed within each instrument and will not be adjusted. Adjustments can only be made to the scan speed, step width, and scan range.

X-ray Generator:	40 kV, 15 mA (Miniflex 600) 30 kV, 15 mA (Miniflex II)
Detector:	D/teX Ultra
Scan mode:	Continuous
Scan speed:	40 deg/min

Step width: 0.02 deg
Scan range: 20 – 140 deg

8 Decision Criteria

Check the location of peaks and peak intensity. Results must fall within the following:

- a. The peak height of the 100% peak should be greater than 10,000 cps.
- b. Peaks (2Θ), all ± 0.15 :
 - 28.42
 - 47.30
 - 56.10
 - 69.17
 - 76.37
 - 88.06
 - 94.97
 - 106.73
 - 114.13
 - 127.57
 - 136.93

If the values lie outside the specified range, verify the instrument parameters outlined in this procedure and re-analyze the silicon standard. If the results are still outside the specified range, contact appropriate instrument support personnel.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

12 Safety

The Rigaku MiniFlex XRD produces X-rays. The unit is equipped with appropriate shielding and electrical interlocks which prevent operation under conditions which would allow the escape of ionizing radiation. These interlocks should never be overridden. All personnel operating the spectrometer are routinely monitored via personal radiation monitors, administered at the unit level and tracked by the Health and Safety Group.

The detector window is comprised of beryllium and is extremely delicate. In the event of damage to the window, the beryllium dust created could pose an acute health hazard. If this occurs, seal the chamber and seek assistance from the Health and Safety Group.

13 References

Manufacturer(s)'s Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	4/25/16	New document, previously existed in the Explosives Unit.
1	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Updated heading in Section 5. Removed unchecking of 'ResidualGraph' in Section 6 e. Added 'appropriate instrument support personnel' to Sections 6 g and 8. Updated 'Instrument Operation and Systems Support' in Section 13 and header.

Approval

Redacted - Signatures on File

Drug Chemistry/
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Date: 09/28/2018

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Date: 09/28/2018

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