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 µg/mL each of Caffeine, Fentanyl, MDEA, Oxycodone, Secobarbital; 40 µg/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 x 10^{-4} torr with reagent gas on at approximately 20%
- NICI analysis - 6 x 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 Chromatogaph

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 µm

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 ± 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 ($PCICH_4$) values for the MSD:

a.	PFDTD Tune:	Mass ± 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 ($NCICH_4$) values for the MSD:

a. PFDTD Tune: Mass ± 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 ± 0.5 m/z:

	EI	PICI
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