Title: Performance Monitoring Protocol (QA/QC) for HS-GC(FID/MS) System (TX-38)

1. Introduction

This document addresses the performance monitoring procedure for headspace-gas chromatographic (HS-GC) systems. This is a type of analytical instrument which is used to analyze volatile chemicals that are often released from evidentiary samples. They must be maintained in such a way as to verify their ability to produce valid results, often for use within judicial proceedings.

2. Scope

This procedure is used to ensure that an instrument is in an operating condition such that samples, can be analyzed accurately, reliably, and in a defensible manner. Quality assurance and quality control (QA/QC) within a forensic laboratory is vitally important and enables results to be trusted. Samples and actions taken to maintain instrumentation are included within this procedure and are related to normal routine casework conducted within the Toxicology Unit.

3. Principle

This procedure involves the daily evaluation of equipment for the use of analyzing samples for the possible presence of volatile compounds, (e.g., methanol, ethanol, acetone, isopropanol, and similar compounds). The term daily refers to each day the instrument is used for analysis of critical samples (e.g., casework). Samples are typically in aqueous environments, are sealed within air-tight vials, and are examined using headspace analysis-type techniques. Volatile components, when placed in closed heated environments, reach an equilibrium between their vapors and their solutions. Vapor phases are analyzed with appropriate chromatographic columns and detectors (e.g., HS-GC(FID/MS), dual column technique).

4. Specimens

Any aqueous sample may be suitable for this analysis. When available, 0.2 mL of specimen is typically used within this procedure.

5. Equipment/Materials/Reagents

5.1 General laboratory glassware (e.g., Class A glassware)

5.2 Headspace autosampler vials (e.g., 20 mL) with appropriate seals/caps and crimper.

5.3 Pipettes (e.g., automatic pipettor-diluter), or equivalent

5.4 Headspace-gas chromatograph with mass spectral detector (HS-GC(FID/MS)), Agilent (or equivalent)

5.5 Perfluorotributylamine (PFTBA, FC-43) (Agilent or equivalent)

5.6 Capillary Column #1: (Rtx-BAC1 ; Restek 18003, 30m, 0.32mm i.d., 1.8 µm, or equivalent)

5.7 Capillary Column #2 (Rtx-BAC2 ; Restek 18002, 30m, 0.32mm i.d., 1.2 µm, or equivalent)

5.8 Deionized water (DIW, Millipore, or equivalent)

5.9 Volatile Mix Performance Solution (MeOH, EtOH, Acetone, IPA ; 0.100 $g\%_{(aq)}$; Certified Reference Material (CRM), Cerilliant (A-127), or equivalent)

6. 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. It is stored in a glass container attached to the MSD. Under normal conditions, this should not need to be refilled.

7. Preparation of Performance Solutions

An externally-prepared ethanol certified reference material (CRM) solution at 0.100 g% will be analyzed for QA/QC instrument evaluation purposes. If these CRM solutions are unavailable, then the Lead Examiner (or higher) will be notified and, upon written approval, equivalent QA/QC performance (e.g., Internally/In-House prepared) solutions can be used. If substitutions are used, then these will be documented within appropriate instrument logbooks.

With the exception of the negative control and blanks, externally-prepared solutions will primarily be used for QA/QC purposes. A typical daily QA/QC sequence will include the following:

a) Negative Control (contains DIW and internal standard)

b) Externally-Prepared Volatile Mix [CRM] Performance Solution (0.100 g% ; MeOH, EtOH, Acetone, IPA)

c) Water Blank (without internal standard)

8. Procedure

8.1 Record the remaining disk space from the instrument's hard drive. Do not use if less than 100 MB of disk space remain. If not recently performed, data backups should occur at the beginning of each month.

8.2 Check the time and date on both the computer (and instrument, if applicable) to ensure they are correct.

8.3 Check the line pressure of the helium supply (carrier gas). Change the tank if less than 100 psi remains.

8.4 Check hydrogen pressure and ensure water level is appropriate within generator. Add deionized water, if needed.

8.5 Ensure that the detectors are operating (i.e., FID is lit and perform an autotune or tune evaluation on the MSD using ATUNE). Save a print out of the autotune or tune evaluation results, and place within the appropriate instrument logbook.

8.5.1 A Tune Evaluation will be performed before starting any QA/QC on the instrument. Evalute all results on the ouput and determine if all results are reported as "OK"

8.5.2 An autoune is performed after MSD maintainence or troubleshooting. A tune evaluation will be peformed after an autotune

8.5.2.1 Example of MSD maintenance include: Replacing a column, cleaning the ion source, refilling PFTBA calibration vial, replacing carrier gas filter or similar.

8.5.3 Mass Spectral Tune:

The following are typical ATUNE values for the MSD (these parameters are evaluated as part of the tune evaluation performed):

8.5.3.1 PFTBA Tune: Mass ±0.2 for m/z 69, 219, and 502

8.5.3.2 Peak width (Pw50): 0.6±0.05 amu (0.55-0.65)

8.5.3.3 Isotope Ratios:
70: 69 (0.5%–1.6%)
220: 219 (3.2%–5.4%)
503: 502 (7.9%–12.3%)

8.5.3.4 Relative abundance:
69 equal to 100 %
219 greater than 45 %
502 greater than 2 %

8.5.3.5 Total number of peaks should be less than 300

8.5.3.6 Air/Water:

Water <20% (shown on tune evaluation report as ratio of 18 to 69)

Nitrogen <10% (shown on tune evaluation report as ratio of 28 to 69)

Oxygen <10%

8.6 Place all solutions within a room temperature environment for approximately ten (10) minutes.

8.7 Prepare or acquire necessary performance solutions.

8.7.1 Label vials appropriately.

8.7.2 Individually add 200 µL of sample and 1 mL of internal standard into separate autosampler vials and cap.

Note: While using the pipette/Diluter is preferred, manual pipetting is acceptable.

Note: The QA/QC instrument performance must be evaluated on a daily basis and prior to samples being analyzed. If sequences continue into the next day, then the current sequence need not be interrupted.

However, a new QA/QC evaluation will begin prior to a new sequence/batch being analyzed. Additional QA/QC analyses may be performed more frequently.

Setting-up Instrument for QC/QC Analysis Analysis:

8.8 Ensure air and hydrogen are flowing and ensure detectors (flame ionization detector (FID)) and/or mass spectrometer (MS) are operational.

8.9 Prepare the sequence table. Record lot number for the 0.100g% CRM within the sample name in the sequence.

8.10 Place the labeled headspace vials in the appropriate order within the instrument.

8.11 Save the sequence/Batch Table using the day's date and the analyst's initials in the appropriate QCQC folder (e.g., AB-02-22-2022_QAQC).

8.12 Ensure there's a printout of the instrument method in the logbook. Evaluate that method printout from the logbook, with the one which is ready to be used in the sequence, to ensure that all parameters are correct.

8.13 Begin the sequence, analyze the samples, and evaluate the data.

8.14 Place the data printouts for the negative control, CRM and blank sample into the logbook.

8.15 Update the logbook with results of the instrument evaluation. Notify the lead Examiner (or higher), if anything fails that can't be fixed.
Record all problems and resolutions within the logbook.

9. Instrumentation

Instrumental Parameters:

HS-GC(FID/MS):

The following parameters can be used. Some values may change due to slight variability (e.g., flow rates). Significant differences must be approved by Unit Lead (or higher) prior to changing. Documentation of such changes must be included with batch data, so that any instrumental parameter change can be associated with data and casework, until this procedure has been updated.

Autosampler	Parameters		Autosampler	Parameters
GC Cycle Time	6.5 min.		Sample Vial Penetration	15 mm
Sample Volume	0.5 mL		Sample Vial Penet. Speed	50 mm/sec
Incubation Time	5 min.		Sample Aspiration Rate	12 mL/min
Incubation Time Increment	0 min.		Sample Post Asp. Delay	1 sec.
Heat Agitator	On		Inlet Penetration Depth	45 mm
Incubation Temperature	50 C		Inlet Penet. Speed	50 mm/sec
Heat Syringe	On		Pre-Inject Time Delay	0.5 sec.
Pre-Injection Flush Time	5 sec.		Inject Flow Rate	10 mL/min.
Agitator Speed	250 rpm		Post Injection Delay	0.5 sec
Agitator On Time	5 sec.		Flush Time	10 sec.
Agitator Off Time	5 sec.		Continuous Flush	Off
GC	**Parameters**		**Injector**	**Parameters**
Run Time	6.5 min.		Mode	SPLIT
Post Run Time	0 min.		Split Ratio	10:1
Oven Equilibration Time	0 min.		Split Flow	46.049 mL/min.
Max. Oven Temperature	250 C		Injector Heater	250 C
Initial Temperature	47 C		Injector Pressure	9.5 psi
Hold Time	6.5 min.		Injector Total Flow	53.654 mL/min.
Post Run Temperature	100 C		Septum Purge Flow	3 mL/min.
			Gas Saver	On
Transfer Line	**Parameters**			
Temperature	200 C			

HS-GC(FID/MS) (Cont'd):

Column Information	**Agilent**	**Agilent**
Column Names	DB-BAC-1 Ultra Inert (Col. #2)	DB-BAC-2 Ultra Inert (Col. #3)
Column Cat. #	123-9334UI	123-9434UI
Film Thickness	1.8 µm	1.2 µm
Column Length	30 m	30 m
Inner Diameter	320 µm	320 µm
Column Max Temp	260°C	260°C
Pressure (initial)	7.9 psi	7.9 psi
Pressure (post run)	3.8589	3.8589 psi
Flow	2.6857 mL/min.	1.5733 mL/min.
Ave. Velocity	59.637 cm/sec.	27.599 cm/sec.
Holdup Time	0.8384 min.	1.8117 min.
Detector	**MS**	**FID (Front Detector)**
Temperature (Source)	230 C	250 C
Temperature (Quad)	150 C	–
Sampling Rate	–	20 Hz (Signal #1)
Makeup Gas	–	He
Makeup Flow	–	25 mL/min.
H_2 Flow	–	30 mL/min.
Air Flow	–	400 mL/min.
Ion Source	EI	–
Fixed Electron Energy	70 eV	–
Solvent Delay	1 min.	–
Gain Factor	2	–
EM Saver	False	–
Acquisition Type	Scan	–
Start Mass	29 m/z	–
End Mass	200 m/z	–
Threshold	0	–
Scan Speed	781 (N=3) Scan Speed	–

10. Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this procedure. Retention time (chromatographic characteristic) and peak shape are used as the basis for peak detection. In most cases all of the criteria below will be met in order to ensure instrument operability for casework.

10.1 Chromatography:

Chromatographic peaks will possess good chromatographic quality (i.e., Gaussian peak shape, reasonable peak width, distinguishing signal-to-noise). In order for a chromatographic peak to be deemed acceptable, it will compare favorably to corresponding chromatographic peaks from the previous day's successful QA/QC evaluation.

10.1.1 Retention Time (RT):

Retention times of chromatographic peaks of interest should compare favorably (i.e., be within ±0.1 minute) to retention times of related peaks from the previous day's successful QA/QC evaluation.

10.1.2 Acceptability of Chromatographic Data:

Chromatographic peaks must be present for all four (4) analytes within the 0.100 g% Volatile Mix [CRM] Performance Solution. The relative areas for each component (MeOH, EtOH, IPA, Acetone) should be greater than 0.35, 0.90, 2.5, 7.5, respectively. Using the 'Percent Report' function can assist with obtaining this information.

Note: Relative areas for analytes are analyte chromatographic peak areas divided by the internal standard peak area. These areas can either me automatically or manually integrated.

10.1.3 Mass Spectra:

No significant peaks of analytes not expected within QA/QC solutions will be present. Significant peaks are considered peaks which have been automatically integrated and/or which negatively affect the quality of the results.

11. Safety

This procedure is carried out in a laboratory environment, and standard safety procedures appropriate for such an environment will be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). Potentially contaminated items and surfaces will be cleaned prior to use.

12. References

Manufacturer's Instrument Manuals

Li-Hua, Xu and Vargas, Carolos, *Reviewing the Autotune Report for Agilent 5977 GC/MSD System in EI Mode* (2021)