
Toxicology Gas Chromatography-Mass Spectrometry (GC-MS)

1.0 Purpose - This procedure specifies the required elements for the calibration and use of the Agilent Gas Chromatograph interfaced to the Agilent 5973 or 5975 series MSD for Toxicology analyses.

2.0 Scope – This procedure applies to all GC-MS instruments used for toxicology analyses in the Toxicology Units of the State Crime Laboratory.

3.0 Definitions

- **Performance verification** – The initial confirmation of the reliability of a previously or externally validated method or instrument.
- **Probability Based Matching (PBM)** - An algorithm designed to compare an unknown mass spectrum against a reference collection of mass spectra for the purpose of identification.
- **Quality control (QC) check** – Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Agilent Gas Chromatograph 6890 or 7890 (GC) equipped with automatic liquid sampler, PC with Agilent Analytical MSD Productivity ChemStation software or equivalent, printer or other output device, merlin microseal, 10 µL syringe and DB5-MS column, 30 m X 0.250 mm X 0.25 µm or DB5-MS Column, 12 m X 0.200 mm X 0.33 µm, or other column as needed.
- Agilent 5973 or 5975 Series Mass Selective Detector (MSD)
- Sample vials and caps

4.2 Materials

- Sample vials and caps

4.3 Commercial Reagents

- Methanol, ACS grade
- Acetone, ACS grade
- Methylene Chloride, ACS grade
- Ethyl Acetate, ACS grade
- Helium Gas, Grade 5.0
- Perfluorotributylamine (PFTBA), neat

4.4 Reference Material Standards

- Multi-component drug solutions
- Prazepam
- Methohexital
- Nalorphine
- d-11 Amphetamine
- d-11 Methamphetamine

5.0 Procedure

5.1 Instrument Performance Verification for New Instrumentation

- 5.1.1** New Toxicology GC-MS instruments shall be installed by a manufacturer representative and shown to meet manufacturer requirements.
- 5.1.2** The Toxicology GC-MS Key Operator or designee shall conduct performance verification on new GC-MS instruments prior to use for casework.
- 5.1.2.1** Performance verification shall include successful tunes (see **5.3**) on three separate days.
 - 5.1.2.2** The performance verification shall include a multi-component reference material standard solution containing the appropriate internal standard run on three separate days. The mass spectra of each component shall be successfully compared to Reference Material, the components shall be visually baseline resolved and the relative retention times of each component shall not vary more than 2.0 % from the standard relative retention times.
 - 5.1.2.3** The data shall be filed and maintained by the Toxicology GC-MS Key Operator to document the new instrument set up.
 - 5.1.2.4** A new entry for the instrument shall be made in the Resource Manager section of FA prior to use in casework. The new entry shall include the following:
 - 5.1.2.4.1** Manufacturer's serial number.
 - 5.1.2.4.2** Unique section identifier for the new instrument.
 - 5.1.2.4.3** Notation under "Verification Date" to reflect the date the performance verification was completed.

5.2 Maintenance

- 5.2.1** Record all maintenance in the instrument log at the time it is performed.
- 5.2.2** Record lengths of column trimmed during maintenance. If the column is trimmed, the instrument shall be out of service until a post-maintenance check is successfully performed.
- 5.2.2.1** If the column is trimmed, a post-maintenance check shall be performed. The check shall include analyzing a multi-component reference material standard solution containing the appropriate internal standard. The relative retention times shall not vary more than 2.0 % from the standard relative retention times.

5.2.2.2 The Toxicology GC-MS Key Operator or designee shall update the instrument log when the instrument is ready to be used for casework and file any generated data in the instrument notebook located near the instrument.

5.2.3 Routine Maintenance - The routine maintenance schedule is a suggested minimum guideline. The maintenance schedule will be determined by the Toxicology GC-MS Key Operator or designee based upon instrument use and performance.

5.2.3.1 Wash Vials

- Rinse and/or fill with the appropriate solvent daily when in use.
- Post-maintenance check: None.

5.2.3.2 Liner

- Replace weekly, prior to the start of the first sequence each week
- Post-maintenance check: Successful tune (see **5.3**).
- Post-maintenance check: Analyze a multi-component reference material standard solution containing internal standard. The relative retention times shall not vary more than 2.0 % from the standard relative retention times.

5.2.3.3 Syringe

- Inspect monthly for cleanliness and ease of movement. Replace as needed.
- Post-maintenance check: Analyze a multi-component reference material standard solution containing internal standard. The relative retention times shall not vary more than 2.0 % from the standard relative retention times.

5.2.3.4 Pump Oil

- Change every six months.
- Post-maintenance check: Successful tune (see **5.3**).

5.2.3.5 Clean Source

- Clean annually.
- Post-maintenance check: Successful tune (see **5.3**).

5.2.3.6 Gold Seal

- Replace annually.
- Post-maintenance check: Successful tune (see **5.3**).
- Post-maintenance check: Analyze a multi-component reference material standard solution containing internal standard. The relative retention times shall not vary more than 2.0 % from the standard relative retention times.

5.2.4 Non-routine Maintenance

5.2.4.1 When non-routine maintenance is performed, the instrument shall be out of service until the non-routine maintenance is evaluated by the Toxicology GC-MS Key Operator or designee to determine the need for additional instrument checks or recalibration prior to analyzing samples.

5.2.4.1.1 If the maintenance may affect chromatography or retention times, a post-maintenance check shall be performed. The check shall include analyzing a multi-component reference material standard solution containing the appropriate internal standard. The relative retention times of a minimum of two components shall not vary more than 2.0 % from the standard relative retention times.

5.2.4.2 The Toxicology GC-MS Key Operator or designee shall update the instrument log when the instrument is ready to be used for casework and file any generated data in the instrument notebook located near the instrument.

5.2.5 Shutdown

5.2.5.1 A successful (see **5.3**) tune shall be performed following any GC or MS shutdown.

5.2.5.2 The shutdown shall be noted in the maintenance log.

5.3 Calibrations (Tune) – MSD

5.3.1 Calibration (tuning) shall be completed successfully prior to beginning the first sample sequence each day. Sample sequences that continue overnight may be allowed to complete without performing a new tune provided that they do not extend more than twenty-four hours beyond the time of the tune. Exceptions to this requirement must have prior approval of the Toxicology Technical Leader.

5.3.2 Perform the Autotune (atune) with Perfluorotributylamine (PFTBA) as the tuning standard.

5.3.3 Compare the Autotune report to previous ones and notify the Toxicology GC-MS Key Operator or designee of any major variations.

5.3.4 The mass assignments of the three tuning masses in the upper part of the report shall be within +/- 0.2 amu of 69.00, 219.00, and 502.00. If the deviation is larger than +/- 0.2 amu, document the deviation on the tune and in the activity log. Perform another autotune. If the problem persists document the deviation on the tune and in the activity log and notify the Toxicology GC-MS Key Operator or designee. The instrument shall remain out of service until the problem is corrected.

5.3.5 The peak widths of the three tuning masses shall be 0.60 +/- 0.10 amu and the peaks shall generally be smooth and symmetrical. If the deviation is greater than 0.10 amu, document the deviation on the tune and in the activity log. Perform another autotune. If the problem persists document the deviation on the tune and in the activity log and notify the Toxicology GC-MS Key Operator or designee. The instrument shall remain out of service until the problem is corrected.

- 5.3.6** The 70/69 isotopic ratio shall be from 0.5 – 1.6, the 220/219 ratio shall be from 3.2 – 5.4, and the 503/502 the ratio shall be from 7.9 – 12.3. If these requirements are not met, document the deviation on the tune and in the activity log. Perform another autotune. If the problem persists, document the deviation on the tune and in the activity log and notify the Toxicology GC-MS Key Operator or designee. The instrument shall remain out of service until the problem is corrected.
- 5.3.7** The abundance of any peaks less than 69 amu shall not be greater than 10 % of the abundance of the base peak.
- 5.3.7.1** Peaks at 18, 28 or 32 amu are indicative of water, nitrogen and oxygen, respectively, and may indicate an air leak.
- 5.3.7.2** If an air leak is detected, the air leak shall be isolated and corrected and the tune repeated. Place the tunes in the logbook. Record the maintenance activity in the activity log and the maintenance log. If the problem persists, document the deviation on the tune and in the activity log and notify the Toxicology GC-MS Key Operator or designee. The instrument shall remain out of service until the problem is corrected.
- 5.3.8** Record each tune in the instrument log along with initials and date and any parameters that are out of specification.
- 5.3.9** Initial the tune report and mark any parameters that are out of specification. File the tune report in the tune section of the logbook.

5.4 Standards and Controls

- 5.4.1** Internal standards, positive and/or negative controls are detailed in the Drug Chemistry Section Toxicology Unit technical procedure used for sample preparation.
- 5.4.1.1** All GC-MS sequences involving case samples will include an injection of the negative and positive control at the beginning and end of each sequence.
- 5.4.2 Blank injections**
- 5.4.2.1** Prior to the injection of a sample, a blank solvent injection shall be made using the same method as the sample.
- 5.4.2.2** The blank solvent injection shall be evaluated to ensure that the instrument is free of any substance being identified in the subsequent sample. The presence of large amounts of common gas chromatography peaks (i.e., siloxanes) shall be noted in the instrument log and reported to the Toxicology GC-MS Key Operator or designee.
- 5.4.3 Syringe flush**
- 5.4.3.1** The syringe shall be flushed at least 10 times with each wash solvent between injections to ensure the sample integrity between injections and that no sample transfer is made between sample vials.

5.4.3.2 Ethyl acetate shall be used in the first wash vial.

5.4.3.3 Methanol shall be used in the second wash vial.

5.5 Sampling

5.5.1 Refer to the Toxicology Unit technical procedure used for sample preparation.

5.6 Instrument Procedure

5.6.1 If an instrument problem or error message occurs, the Forensic Scientist who discovers the problem shall document the problem in the activity log. If the problem cannot be corrected immediately, the Forensic Scientist shall mark the activity log to show that the instrument is out of service, notify the Toxicology GC-MS Key Operator or designee and notify all other Forensic Scientists affected.

5.6.2 A logbook shall be maintained near each instrument.

5.6.3 The logbook shall contain a GC-MS Log.

5.6.3.1 The GC-MS log shall contain the date, sequence name, initials of operator, and comments.

5.6.3.2 The GC-MS log shall contain the date of maintenance, description of maintenance performed, length of any column trimmed, parts replaced, and the initials of the person performing or documenting the maintenance.

5.6.3.3 Any unusual error messages shall be recorded in the GC-MS log.

5.6.4 Upon completion of a sequence, the "Sequence Log" shall be printed and stored in the logbook.

5.6.5 The logbook shall contain the tune reports. Tunes performed to check instrument performance during maintenance or troubleshooting need not be retained.

5.6.6 The logbook shall contain any post maintenance data generated.

5.6.7 The logbook shall be archived yearly and labeled with the instrument serial number and year. The archived logbook shall be stored near the instrument.

5.6.8 Sequences

5.6.8.1 The current date shall be used in name of a sequence. Upon completion of a sequence, the sequence log shall be stored in the instrument logbook.

5.6.9 Data Files

5.6.9.1 Data file names shall include the year designation and the case file number to ensure that files from different years with the same file number are distinguishable.

5.6.9.2 Data files associated with casework shall not be deleted or overwritten.

5.6.9.3 Data shall be archived annually and labeled with the instrument serial number and dates. Notify the Toxicology GC-MS Key Operator or designee if the disk drive(s) become full.

5.6.10 For quantitative methods refer to the Toxicology Unit technical procedure used for sample preparation for data analysis, identification and reporting.

5.6.11 Evaluate the chromatogram and spectra for peaks of interest using the criteria listed in **5.6.12**.

5.6.12 Identification for Qualitative Methods

5.6.12.1 The GC-MS provides retention time data and mass spectral data.

5.6.12.2 Mass Spectral Identification

5.6.12.2.1 The mass spectrum shall be searched and compared to a reference collection of reference material mass spectra. Probability Based Matching (PBM) shall be used to aid the analyst in the identification but shall not be used as the sole basis of the identification.

5.6.12.2.2 The mass spectrum must contain all of the major and diagnostic ions unique to the analyte.

5.6.12.2.3 All ions with a relative intensity greater than 10 % of the base peak in the reference standard spectrum must be present in the sample spectrum.

5.6.12.2.4 The presence of additional major ions in the mass spectrum is indicative of background noise or a co-eluting substance. Isolate the source of the additional ions and subtract prior to searching the reference collection of reference material mass spectra.

5.6.12.2.5 The case record shall contain the mass spectrum of the reference material standard with a Drug Chemistry vault ID or supplier/lot number or other appropriate Drug Chemistry Designation.

5.6.12.3 Chromatographic Criteria

5.6.12.3.1 The signal to noise ratio of the internal standard(s) must be greater than 5:1. The signal to noise ratio is defined as the response at the baseline or valley immediately before the internal standard signal.

5.6.12.3.2 The relative retention time compared to a reference material standard shall have a difference of 2.0 % or less. The standard relative retention time shall be determined under the same chromatographic conditions as the sample.

5.6.13 Reporting for Qualitative Methods

5.6.13.1 For an analyte to be reported, it must meet all the acceptance criteria in **5.6.12** and not be identified in the negative control or the corresponding blank.

5.6.13.2 Refer to the Toxicology Unit [Technical Procedure for Toxicology Analysis](#) for reporting.

5.7 Calculations

5.7.1 RRT Calculation: (analyte retention time / internal standard retention time) rounded to the nearest thousandth. A retention time may be determined as the elution time at which the mass spectrum was collected or with an integrator in the ChemStation software.

5.7.2 Percent Difference Calculation:
$$(\text{standard RRT} - \text{analyte RRT}) / (\text{standard RRT}) * 100$$

5.8 Uncertainty of Measurement – N/A

6.0 Limitations

6.1 The GC-MS methods described in this procedure shall not be used to distinguish between optical isomers.

6.2 Introduction of improperly prepared samples may lead to poor sensitivity and carryover.

7.0 Safety

7.1 Refer to the State Crime Laboratory Safety Manual.

7.2 Handle syringes with care to avoid punctures.

7.3 Use extreme caution dismantling/installing/transporting compressed gas cylinders. Cylinders shall not be moved without the cylinder cap securely in place.

7.4 Gas Chromatograph and Mass Spectrometer may be extremely hot. Avoid touching hot areas and wear protective gloves while performing maintenance.

8.0 References

Moffat, A.C., et al., eds. *Clarke's Isolation and Identification of Drugs*, 2nd Edition. London: Pharmaceutical Press, 1986.

Skoog, Douglas A., James Hollar and Timothy A. Nieman. *Principles of Instrumental Analysis*, 5th Ed., Garcourt Brace & Company, 1998.

Agilent GC-MSD ChemStation and Instrument Operation Student Manual Course Number H4043A Volume 1, Revision E.02.xx. Agilent Technologies, February 2008.

Pfleger, Maurer, and Weber. *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites*. 2nd. Ed., Vols. 1-3, 1992.

Agilent 6890 GC Instrument Manuals.

Agilent 5973 and 5975 Instrument Manuals.

9.0 Records

- GC-MS logbook
- GC-MS log

10.0 Attachments- N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Technical Procedure J-16 converted to ISO standards
10/26/2012	2	5.3.6 - removed; 5.6.8 - all methods had the phrase “or equivalent” added to column description; 5.6.8.5 and 5.6.12 - removed CANSIMFS; 5.7.1 - changed hundredth to thousandth; 5.7.2 - removed round to one decimal place; grammar
02/08/2013	3	2.0 - modified for procedure consolidation 4.1 - added equipment 5.6.8 - removed section for procedure consolidation 5.1.2.2, 5.6.10, 5.6.11 - removed reference to specific names of instrumental methods
05/03/2013	4	3.0 - added definition 5.6.12.2 - reworded and inserted additional criteria 5.6.13.1 - corrected reference to acceptance criteria
11/15/2013	5	Added issuing authority to header
05/09/2014	6	4.4 – Removed references to Hexobarbital and Phenobarbital-d5. Added d-11 Amphetamine and d-11 Methamphetamine. 5.2.3.2 - Changed time frame for liner change and added criteria for post maintenance check 5.2.3.3, 5.2.3.6 – Added criteria for post maintenance check. 5.4.1.1 – Added requirement to run positive control. 5.4.2.2 – Updated wording. 5.6.11- Added criteria Removed 5.6.11.1 5.6.12.3 – Added and combined chromatographic and RRT criteria. 5.6.13.1 – Added additional reporting criteria 8.0 – Removed referenced articles regarding cannabinoids and phenethylamines