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 Toxicology in the Raleigh, Triad, and Western locations 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.
- **Performance Check –** A test used to verify acceptable system performance.
- **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 or 5 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

- Alprazolam
- Carbamazepine
- Carisoprodol
- Chlorpheniramine
- Mepivacaine

4.5 Prepared Standard - Prepared solutions may be prepared in any amount provided that the component ratios are kept constant.

4.5.1 Performance Check Standard

4.5.1.1 Prepare a solution containing the following reference standards, in ethyl acetate:

- Mepivacaine - 20 µg/mL
- Carbamazepine - 20 µg/mL
- Carisoprodol - 20 µg/mL
- Chlorpheniramine - 4 µg/mL
- Alprazolam - 4 µg/mL

4.5.1.1.1 Example – To a 10 mL volumetric flask, add 0.200 mL of 1mg/mL mepivacaine, 0.200 mL of 1mg/mL carbamazepine, 0.200 mL of 1mg/mL carisoprodol, 0.040 mL of chlorpheniramine, and 0.040 mL of alprazolam. Fill the flask to the volumetric mark.

4.5.1.2 Lot number: Eight digit format year/month/day.

4.5.1.1.1 Example: 20101231

4.5.1.3 Expiration: One year from date of preparation.

4.5.1.4 Store in a freezer.

4.5.1.5 QC check: analyze the standard by GC-MS. The mass spectrum of each component shall be compared successfully to Reference Material, the components shall be baseline resolved visually and the relative retention times of each component shall not vary more than 2.0 % from the standard relative retention times.

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 the analysis of the Performance Check Standard run on three separate days. The mass spectrum of each component shall be successfully compared to Reference Material, the components shall be baseline resolved visually 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 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:

5.1.2.3.1 Manufacturer's serial number.

5.1.2.3.2 Unique section identifier for the new instrument.

5.1.2.3.3 Notation under "Verification Date" to reflect the date the performance verification was completed.

5.1.2.3.4 The data generated during the performance verification for a new GC-MS. (The file shall be approved in FA by the GC-MS Key Operator or Toxicology Technical Leader.)

5.2 Performance Check of the GC-MS System

5.2.1 A performance check is performed by the analysis of the Performance Check Standard Solution using the appropriate instrumental method

5.2.1.1 The relative retention times of all components relative to Mepivacaine shall not vary more than 2.0 % from the standard relative retention times.

5.3 Maintenance

5.3.1 Record all maintenance in the instrument log at the time it is performed.

5.3.2 Record lengths of column trimmed during maintenance.

5.3.3 After any maintenance, the instrument shall be labeled as being out of service until a post-maintenance check is performed successfully.

5.3.3.1 The Toxicology GC-MS Key Operator or designee shall update the instrument log when the instrument is ready to be used for casework, by indicating the post maintenance checks were successful. Generated raw data shall be stored in the instrument's computer folder where the raw data file is stored.

5.3.4 Routine Maintenance

5.3.4.1 Wash Vials

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

5.3.4.2 Liner

- The liner shall be changed prior to the start of a sequence containing a case sample.

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

5.3.4.3 Syringe

- Inspect monthly for cleanliness and ease of movement. Replace as needed.
- Post-maintenance check: Successful performance check.

5.3.4.4 Pump Oil

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

5.3.4.5 Clean Source

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

5.3.4.6 Gold Seal

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

5.3.4.7 Helium Tank

- Replace as needed to insure a supply of helium.
- Post-maintenance check: Successful tune (see **5.3**).
- Post-maintenance check: Successful performance check.

5.3.5 Non-routine Maintenance

5.3.5.1 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.

5.3.6 Shutdown

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

5.3.6.2 The shutdown shall be noted in the maintenance log.

5.3.7 Performance check sample processed data shall be stored in the object repository for that instrument in Forensic Advantage (FA).

5.4 Calibrations (Tune) – MSD

5.4.1 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.

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

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

5.4.4 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.

5.4.5 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.

5.4.6 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.

5.4.7 The abundance of any peaks less than 69 amu shall not be greater than 10 % of the abundance of the base peak.

5.4.7.1 Peaks at 18, 28 or 32 amu are indicative of water, nitrogen and oxygen, respectively, and may indicate an air leak.

5.4.7.2 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.

5.4.8 Record each tune in the instrument log along with initials and date and any parameters that are out of specification.

5.4.9 Initial the tune report and note any parameters that are out of specification in the GC-MS Log. Electronically generated or scanned tune reports shall be stored in the "Tunes" folder of the instrument's computer. Electronically generated tune reports do not require initials.

5.5 Standards and Controls

5.5.1 Internal standards, positive and/or negative controls are detailed in the Toxicology technical procedure used for sample preparation.

5.5.1.1 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.

5.5.1.2 Positive and negative control sets will be included in the sequence so they occur after every 20 case samples at a minimum.

5.5.1.3 No positive and negative control extract may be injected more than twice.

5.5.2 Blank injections

5.5.2.1 Prior to the injection of a case sample, a blank solvent injection shall be made using the same method as the sample.

5.5.3 Syringe flush

5.5.3.1 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.

5.5.3.2 Ethyl acetate shall be used in the first set of wash vial(s).

5.5.3.3 Methanol shall be used in the second set of wash vial(s).

5.6 Sampling

5.6.1 Refer to the Toxicology technical procedure used for sample preparation.

5.7 Instrument Procedure

5.7.1 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.

5.7.2 A logbook shall be maintained near each instrument.

5.7.3 The logbook shall contain a GC-MS Log.

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

5.7.3.2 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.

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

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

5.7.5 Sequences

5.7.5.1 The current date shall be used in the name of a sequence.

5.7.5.2 For sequences involving case samples, a second person shall verify that the vial placement on the instrument matches what is listed in the sequence.

5.7.5.3 The sequence shall be printed, then initialed and dated by both people.

5.7.5.4 If the sequence is modified after it has been verified, the sequence shall be re-verified as described in 5.7.5.2.

5.7.6 Data Files

5.7.6.1 Data files associated with casework shall include the case file number in the file name or sample name.

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

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.

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
03/20/2015	7	4.4 – added Mepivacaine 5.2.3.1 – made refilling a requirement 5.2.3.2 – reduced liner change schedule to daily 5.3.1 – removed 24 hour tune limit 5.4.3.2 and 5.4.3.3 – made "vial" plural 5.6.9.1 - expanded data naming options 5.6.12.3.1 – clarified signal to noise definition
02/12/2016	8	2.0 – revised for consistency between procedures 3.0 – added definition of performance check 4.1 – added equipment 4.4 – added Mepivacaine, Carbamazepine, Carisoprodol, Chlorpheniramine, and Alprazolam 4.4 – removed Prazepam, Methohexital, Nalorphine, d-11 Amphetamine, d-11 Methamphetamine, since they are referenced in other procedures 4.5 - added 5.1.2.3 – moved to 5.1.2.3.4 and added storage location and approval criteria 5.1.2.2, [5.3.2.1, 5.3.3.2, 5.3.3.3, 5.3.3.6, 5.3.4.1 (old 5.2.2.1, 5.2.3.2, 5.2.3.3, 5.2.3.6, 5.2.4.1)] - changed the reference from "a multi-component reference material standard solution containing the appropriate internal standard" to "the Performance Check Standard." 5.2 – added to consolidate post maintenance requirements 5.3.3 (old 5.2.3) – removed unnecessary language 5.3.4 (old 5.2.4) - removed unnecessary language 5.3.4.2 (old 5.2.4.2) – revised liner replacement criteria 5.3.4.2, 5.3.4.3, 5.3.4.6 (old 5.2.4.2, 5.2.4.3, 5.2.4.6) - removed consolidated requirement which was moved to 5.2. Old 5.2.5.1.1 – removed unnecessary language, and language consolidated in 5.2 5.3.4.7 - new 5.3.2.2 and 5.3.4.2 (old 5.2.2.2 and 5.2.4.2) – removed location of maintenance data storage requirement and put it in 5.2.6 for consistency 5.3.7 - new 5.4.9 (old 5.3.9) – changed data storage location. 5.5.1 and 5.6.1 (old 5.4.1 and 5.5.1) – cleaned up reference to just Toxicology 5.5.1.2 and 5.5.1.3 (old 5.4.1.2 and 5.4.1.3) – new to clarify QC frequency 5.5.2.1 (old 5.4.2.1) – specified "case" sample. 5.5.2.2 (old 5.4.2.2) – moved to new procedure on Mass Spec Data Processing

		5.6.4, 5.6.5, 5.6.6 – removed - data print-out requirement removed 5.7.5.1 (old 5.6.8.1) - data print-out requirement removed 5.7.5.2 - added old 5.6.9.3 – removed old 5.6.11, 5.6.12, 5.6.13, and 5.7 – removed (moved to new procedure Mass Spec Data Processing) 6.2 – removed