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 equivalent 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
- Agilent 5973 or equivalent Mass Selective Detector (MSD)

4.2 Materials

- Sample vials, caps, and inserts
- Merlin microseal, liners
- 10 µL or 5 µL syringe
- DB5-MS column, 30 m X 0.250 mm X 0.25 µm or equivalent, or other column as needed

4.3 Commercial Reagents

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

4.4 Reference Material Standards

- Alprazolam
- Carbamazepine
- Carisoprodol
- Chlorpheniramine
- Mepivacaine
- Trazodone

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
- Trazodone- 10 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 1mg/mL chlorpheniramine, 0.040 mL of 1mg/mL alprazolam, and 0.100 mL of 1mg/mL trazodone. Bring to 10 ml volume with ethyl acetate.

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. See **5.2.1** for acceptance criteria.

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 **5.4**) on three separate days.

5.1.2.2 The performance verification shall include the successful analysis of the Performance Check Standard run on three separate days (see **5.2.1**).

5.1.2.3 A new entry for the instrument shall be made in the Resource Manager section of Forensic Advantage (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.

5.2.1.2 No analyte unrelated to the standards within the Performance Check Standard Solution shall be identified within the Performance Check.

5.2.1.3 All analytes shall meet Mass Spectral Acceptance Criteria as listed in the procedure: GC-MS Data Processing.

5.2.1.4 Performance check sample processed data shall be stored in the object repository for that instrument in FA.

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 in the log as well as the computer software for the instrument.

5.3.3 After any maintenance, the instrument shall be labeled as being out of service until a performance 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 performance check was 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 A tune (see **5.4**) and performance check (see **5.2)** must be performed after all maintenance. A successful tune and performance check is required to bring an instrument back into service.

5.3.4.1.1 Liner

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

5.3.4.1.2 Syringe

- Inspect monthly for cleanliness and ease of movement. Replace as needed.

5.3.4.1.3 Pump Oil

- Change every six months.

5.3.4.1.4 Clean Source

- Clean annually.

5.3.4.1.5 Gold Seal

- Replace annually.

5.3.4.1.6 Helium Tank

- Replace as needed to ensure a supply of helium.

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 tune (see **5.4**) and performance check (see **5.**2) shall be performed following any GC or MS shutdown.

5.3.6.2 The shutdown shall be noted in the maintenance log.

5.4 Calibrations (Tune) – MSD

5.4.1 Calibration (tuning) shall be completed successfully within 24 hours prior to beginning the first sample sequence. 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 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, refer to **5.4.7**.

5.4.4 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, refer to **5.4.7**.

5.4.5 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 refer to **5.4.7**.

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

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

5.4.6.2 If an air leak is detected, the air leak shall be isolated and corrected and the tune repeated. Record the maintenance activity in the activity log and the maintenance log. If the problem persists, refer to 5.4.7.

5.4.7 If a tune parameter does not meet acceptance criteria, document the deviation in the activity log. Perform another autotune. Compare the Tune Report to previous ones. If the problem persists, document the deviation 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 pass/fail of each tune on the tune report and in the instrument log along with initials.

5.4.9 All tunes will be stored electronically in the appropriate FA Instrument Resource.

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 control sample may be injected more than twice in an analytical run.

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 15 times with each wash solvent between injections to ensure the sample integrity between injections and that no sample transfer is made between sample vials.

5.5.3.1.1 Wash vials will be rinsed and filled daily with the appropriate solvent when in use.

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.

5.7.4 The logbook shall be archived yearly and labeled with the instrument serial number and year. The archived logbook shall be scanned and placed in the object repository for that instrument in Forensic Advantage (FA).

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 and 5.7.5.3.

5.7.6 Data Files

5.7.6.1 Data files associated with casework shall include the case file number in the 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 – N/A

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 5975Instrument 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

07/07/17	9	1.0 – updated wording 4.1 – moved materials to 4.2, updated wording, removed sample vials and caps 4.2 – added liners 4.3 – removed acetone and methylene chloride 5.1.2.1, 5.3.4.2, 5.3.4.4, 5.3.4.5, 5.3.4.6, 5.3.4.7, 5.3.6.1 – Corrected reference to tune (5.4) Removed 5.4.3 5.4.3, 5.4.4, 5.4.5, 5.4.6.2 (old 5.4.4, 5.4.5, 5.4.6, 5.4.7.2) – removed unnecessary language and consolidated to new 5.4.7 5.4.8 and 5.4.9 – Removed unnecessary language 5.5.1.3 – Added "in an analytical run" 5.5.3.1 – Changed minimum syringe flushes Removed 5.7.3.3 Removed old 6.0 and 6.1
02/22/19	10	4.2 - added inserts 4.4 - added Trazodone 4.5.1.1 and 4.5.1.1.1 - added Trazodone and concentrations of standards used to make performance check, updated wording, 4.5.1.5 - added performance check acceptance criteria 5.1.2.2 - added "successful" and reference 5.2.1.2, 5.2.1.3, 5.2.1.4 - added 5.2.1.5 - moved from 5.3.7 5.3.2 - added "as well as the computer software" 5.3.3 and 5.3.3.1 updated term "post maintenance" to "performance check" 5.3.4 - restructured, new 5.3.4.1 5.3.6 – added performance check requirement 5.4.1 – updated time criteria 5.4.7 - reworded 5.4.8 – added documentation requirement 5.4.9 – updated storage location requirement 5.5.1.3 updated wording 5.5.3.1.1 – added requirement 5.7.4 changed to storing in FA 5.7.5.4 added "and 5.7.5.3" 5.7.6.1 removed "file name or"