Technical Procedure for Drug Chemistry Gas Chromatograph/Mass Spectrometry (GC-MS)

1.0 Purpose - This procedure specifies the required elements for the calibration and use of the Agilent 6890 GC interfaced to the Agilent 5973 or 5975 Series MSD for Drug Chemistry analyses.

2.0 Scope - This procedure applies to all GC-MS instruments used for drug chemistry analyses in the Drug Chemistry Section of the Raleigh location 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.
- **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 (GC)
- Agilent 5973 or 5975 Series Mass Selective Detector (MSD)
- Agilent Automatic Liquid Sampler
- PC with Agilent Analytical MSD Productivity ChemStation Software or equivalent
- Computer Printer or other data output device

4.2 Materials

- Sample vials and caps
- 10 µL syringe
- DB-5 or DB5-MS Column, 30 m X 0.250 mm X 0.25 µm (or other similar column)

4.3 Commercial Reagents

- Methanol, ACS grade
- Hexane, ACS grade
- Chloroform, ACS grade
- Acetonitrile, ACS grade
- Ethyl acetate, ACS grade
- Helium gas, Grade 5.0
- Perfluorotributylamine [PFTBA], neat

4.4 Reference Materials

- Multi-component drug solutions

5.0 Procedure

5.1 Instrument Performance Verification for New Instrumentation

5.1.1 New GC-MS instruments shall be installed by a manufacturer representative and shown to meet any manufacturer's requirements.

5.1.2 The GC-MS Key Operator or designee shall conduct performance verification on new GC-MS instruments prior to use for casework.

5.1.2.1 The performance verification shall include successful tunes (see **5.4**) on three separate days.

5.1.2.2 The performance verification shall include the multi-component reference material standard solutions from **5.3.1.2** and **5.3.1.3** run on the HIGH and LOW methods, respectively, on three separate days. The mass spectra of each component shall be successfully compared to reference material and the percent difference of the highest and lowest retention times of each component shall be not be greater than 2.0 %.

5.1.2.3 The data shall be filed and maintained by the GC-MS Key Operator to document set up of the new instrument.

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 will include:

5.1.2.4.1 The manufacturer's serial number.

5.1.2.4.2 The unique section identifier for the new instrument.

5.1.2.4.3 A notation under "Verification Date" to reflect the date the performance verification was completed.

5.2 Maintenance

5.2.1 Record all maintenance in the activity log and the maintenance log at the time it is performed.

5.2.2 Record lengths of column trimmed in the activity log and the maintenance log. If the column is trimmed, the instrument shall be out of service until a monthly performance check is successfully completed (see **5.3.1**).

5.2.2.1 Standards ran prior to the column maintenance shall not be used for retention time comparison after the column maintenance.

5.2.2.2 The 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 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 wash solvent daily when in use.
- Post-maintenance check: None.

5.2.3.2 Septum

- Replace weekly when in use.
- Post-maintenance check: Successful tune (see **5.4**).

5.2.3.3 Syringe

- Inspect monthly for cleanliness and ease of movement. Replace as needed.
- Post-maintenance check: None.

5.2.3.4 Liner

- Replace monthly when in use.
- Post-maintenance check: Successful tune (see **5.4**).

5.2.3.5 Pump Oil

- Change every six months.
- Post-maintenance check: Successful tune (see **5.4**).

5.2.3.6 Clean Source

- Clean annually.
- Post-maintenance check: Successful tune (see **5.4**) and monthly performance check (see **5.3.1**).

5.2.3.7 Gold Seal

- Replace annually.
- Post-maintenance check: Successful tune (see **5.4**) and monthly performance check (see **5.3.1**).

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 GC-MS Key Operator or designee to determine the need for additional instrument checks prior to analyzing samples.

5.2.4.1.1 If maintenance is performed that may affect retention times, a monthly performance check (see **5.3.1**) shall be performed before the instrument is placed back in service. Standards run prior to column maintenance shall not be used for retention time comparison after column maintenance.

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

5.2.5 Shutdown

5.2.5.1 A successful tune (see **5.4**) shall be performed following any GC or MS shutdown.

5.2.5.2 The shutdown shall be noted in the maintenance log.

5.3 Standards and Controls

5.3.1 Monthly Performance Check

5.3.1.1 Standard solutions shall be injected on a monthly basis when the instrument is in use to verify instrument performance. The solutions shall, when feasible, be run during the first seven calendar days of each month. Any instrument on which the standard solutions are not run during the first seven days of the month shall be out of service until the standard solutions are successfully run.

5.3.1.2 A multi-component standard solution including methandrostenolone and testosterone propionate shall be run on the HIGH temperature program.

5.3.1.3 A multi-component standard solution including phentermine and methamphetamine, and a multi-component standard solution including codeine and hydrocodone, shall be run on the LOW temperature program.

5.3.1.4 Additional standard solutions may be run on a monthly basis to establish retention times. Any additional monthly standard solutions shall not be required to verify instrument performance.

5.3.1.5 Standard solution data files shall be named with the first letter of each word of the standard solution name followed by the numerical month designation. The corresponding blank shall have a similar name with a designation that it is a blank. Example: The name of an “Amphetamines Mix” standard solution run in January would be “AM01” and the blank would be “AM01-b.”

5.3.1.6 The retention time of each required component of the standard solutions shall be compared to previous runs. Any shift greater than 2.0 % that cannot be attributed to maintenance shall be documented in the instrument log and the instrument evaluated by the GC-MS Key Operator or designee.

5.3.1.7 The mass spectrum of each required component in the standard solution shall be substantially the same as a reference material spectrum. Any appreciable differences shall be noted in the instrument log and the instrument evaluated by the GC-MS Key Operator or designee.

5.3.1.8 The total ion chromatograms for each standard solution shall be visually inspected for resolution between the required components. For the HIGH temperature program, methandrostenolone and testosterone propionate shall be baseline resolved. For the LOW temperature program, phentermine and methamphetamine shall be resolved at a minimum of half-height. Codeine and hydrocodone shall be baseline resolved. Any deficiencies shall be documented in the instrument log and the instrument shall be evaluated by the GC-MS Key Operator or designee.

5.3.1.9 The Forensic Scientist reviewing the required monthly standard solution injections shall print the total ion chromatograms from each standard solution injection with the retention times displayed and the total ion chromatograms of the corresponding blanks. The printouts shall be initialed by the reviewing Forensic Scientist and placed in the "GC RT" section of the instrument notebook. The reviewing Forensic Scientist shall mark the activity log to indicate the successful run of the standard solution.

5.3.2 Blank injections

5.3.2.1 Prior to the injection of a sample, a blank solvent injection shall be made using the same method and split ratio as the sample.

5.3.2.2 The solvent shall be prepared by the individual Forensic Scientist at the time of sample preparation and be the same solvent from the same bottle used in the sample preparation.

5.3.2.3 The blank solvent injection shall be evaluated to ensure that the instrument and solvent are free of any controlled substance, any substance being identified in the sample and any substance that may interfere with the identification of sample component(s). The presence of large amounts of common gas chromatography peaks (e.g., siloxanes) shall be noted in the instrument log and reported to the GC-MS Key Operator or designee.

5.3.3 Syringe flush

5.3.3.1 The syringe shall be flushed at least 10 times with solvent between injections to ensure the sample integrity between injections and to ensure that no sample transfer is made between sample vials.

5.3.3.2 Methanol shall be used in the first wash vial.

5.3.3.3 Hexane or chloroform shall be used in the second wash vial.

5.4 Calibrations (Tune) – MSD

5.4.1 Calibration (tuning) shall be successfully completed 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 or noon, whichever is later.

5.4.2 Perform the Standard Spectra Tune (stune) with Perfluorotributylamine (PFTBA) as the tuning standard.

5.4.3 Compare this Standard Spectra Tune report to previous ones and notify the GC-MS Key Operator of 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 standard spectra tune. If the problem persists document the deviation on the tune and in the activity log and notify the 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 0.55 +/- 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 standard spectra tune. If the problem persists document the deviation on the tune and in the activity log and notify the GC-MS Key Operator or designee. The instrument shall remain out of service until the problem is corrected.

5.4.6 The base peak shall be identified as mass 69. The relative abundance ratio of mass 219 to mass 69 shall be within 40 – 85 % and the relative abundance ratio of mass 502 to mass 69 shall be within 2.0 – 5 %. If these requirements are not met, document the deviation on the tune and in the activity log. Perform another standard spectra tune. If the problem persists, document the deviation on the tune and in the activity log and notify the GC-MS Key Operator or designee. The instrument shall remain out of service until the problem is corrected.

5.4.7 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 standard spectra tune. If the problem persists document the deviation on the tune and in the activity log and notify the GC-MS Key Operator or designee. The instrument shall remain out of service until the problem is corrected.

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

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

5.4.8.2 If an air leak is detected, the air leak shall be isolated and corrected and the tune repeated. Record the tunes and maintenance activity in the instrument logbook. If the problem persists, document the deviation on the tune and in the activity log and notify the GC-MS Key Operator or designee. The instrument shall remain out of service until the problem is corrected.

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

5.4.10 Initial the tune report and mark any parameters that are out of specification. File the tune report in the tune section of the logbook.

5.5 Sampling – Refer to the Drug Chemistry Section Technical Procedure for Drug Chemistry Analysis.

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 immediately corrected, the Forensic Scientist shall mark the activity log to show that the instrument is out of service, notify the GC-MS Key Operator or designee and notify all other Forensic Scientists affected.

5.6.2 Logbook

5.6.2.1 A logbook shall be maintained near each instrument.

5.6.2.2 The logbook shall contain the Activity Log (see Section Files for Printable version).

5.6.2.2.1 The Activity Log shall include the date, sample identification, initials of operator, GC-MS method used, and comments for each sample analyzed. The log shall also include substances observed in the sample.

5.6.2.2.2 Septum changes and any unusual error messages shall be recorded in this section.

5.6.2.2.3 If samples are rerun for any reason, a new entry shall be recorded in the Activity Log. (Blank solvent runs do not need to be recorded.)

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

5.6.2.4 The logbook shall contain the Maintenance Log (see Section Files for printable version). The Maintenance Log shall include the date, description of work performed, length of any column trimmed, parts replaced, and the initials of the person performing or documenting the maintenance. (Septum changes need not be logged in the maintenance section. These entries shall be recorded in the Log section as outlined above.)

5.6.2.5 The logbook shall contain the monthly performance check data. Other retention time reference material data may also be stored in the logbook.

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

5.6.3 Sample Preparation

5.6.3.1 Refer to the Drug Chemistry Section Technical Procedure for Extractions and Separations.

5.6.3.2 Evaluate and prepare samples prior to injection to avoid overloading and the introduction of extreme pH, oil, sugar and compounds known to be retained in the instrument.

5.6.3.3 Solid samples shall be filtered with solvent to prevent particulate matter and undesired compounds from being introduced into the instrument (e.g., sugars). Particulate matter shall not be visible in an autosampler vial.

5.6.3.4 Sulfates shall be extracted/converted prior to introduction into the instrument. Refer to the Drug Chemistry Section Technical Procedure for Extractions and Separations.

5.6.3.5 Derivatizing agents may be used when needed.

5.6.4 GC-MS Methods

5.6.4.1 When the standard methods are not appropriate to analyze a compound, a modified method may be used in accordance with the Laboratory Procedure for Authorizing Deviations.

5.6.4.2 HIGH – typically used for compounds that elute after 13 minutes in the screen method (e.g., buprenorphine, LSD, some steroids and some synthetic cannabinoids).

- 1.0 minute initial time
- 280 °C initial temperature
- 10 °C/minute
- 300 °C final temperature
- 22.0 minutes final time
- 25.0 minutes total time
- Scan range = 40-500 amu

5.6.4.3 LOW – typically used for compounds that elute prior to 13 minutes in the screen method (e.g. cocaine, amphetamines, most opiates and benzodiazepines).

- 1.5 minutes initial time
- 100 °C initial temperature
- 30 °C/minute
- 300 °C final temperature
- 4.83 minutes final time
- 13.0 minutes total time
- Scan range = 40-500 amu

5.6.4.4 SCRN(Screen) – This method shall be used when GC-MS is used to screen samples to determine if a controlled substance may be present. This method shall be used when a controlled substance is not previously indicated and a GC-MS analysis is performed (i.e. negative preliminary testing and/or infrared analysis indicated a non-controlled substance and a GC-MS analysis is performed). This method shall be used when GC-MS is the sole technique used in analysis.

- 1.5 minutes initial time
- 100 °C initial temperature
- 30 °C/minute
- 300 °C final temperature
- 26.83 minutes final time
- 35.0 minutes total time
- Scan range = 40-500 amu

5.6.4.5 Each method may be used with split ratios of 5:1, 20:1, or 100:1. Numbers or an abbreviation behind the method name indicates the split ratio.

5.6.4.6 Splitless injections are generally not utilized, but may be used for sample solutions that did not provide successful identification of a compound using a 5:1 or higher split ratio.

5.6.5 Sequences

5.6.5.1 The current date shall be used in name of a sequence. Sequences need not be archived.

5.6.6 Data Files

5.6.6.1 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.

5.6.6.2 Data files associated with casework and performance checks shall not be deleted or overwritten.

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

5.6.7 Evaluate the chromatogram and spectra for peaks of interest.

5.6.8 The case record shall contain the:

- Total Ion Chromatogram (TIC) for the corresponding blank.
- Total Ion Chromatogram (TIC) for the sample.
- Mass spectra of peaks of interest.
- Expanded mass spectra of phenethylamines.

5.6.9 Identification

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

5.6.9.2 Mass Spectral Identification

5.6.9.2.1 The requirement for mass spectral identification is a mass spectrum that is substantially the same as that of a reference material standard.

5.6.9.2.2 The Case Record shall contain the mass spectrum of the reference material standard with Drug Chemistry vault ID or supplier / lot number or other Drug Chemistry Designation. (Library Search results may be included.)

5.6.9.3 Retention Time (RT) Identification

5.6.9.3.1 The requirement for retention time identification shall be a retention time that when compared to a material standard has a difference by 0.1 minute or less. The retention time may be determined by using an integrator in the Chemstation software

or may be determined as the elution time at which the mass spectrum was collected.

5.6.9.3.2 The reference material standard shall be run within thirty days before or after the case sample. If the reference material standard is a component of a monthly standard solution, then the retention time may be used for the month in which it was run plus the first seven calendar days of the following month. The interval between a sample and a standard injection shall not contain column maintenance.

5.6.9.3.3 The case record shall contain the following:

- Total Ion Chromatogram for the corresponding reference material standard blank.
- Total Ion Chromatogram for the reference material standard. If an integrator is used, the retention times shall be displayed.
- Mass spectrum of the reference material standard and other peaks of interest. If an integrator is not used, the mass spectrum shall display the elution time at which the mass spectrum was collected.
- Reference material standard Drug Chemistry vault ID or supplier/lot number or other Drug Chemistry designation.
- Difference of the standard and sample retention time.

5.6.10 **Reporting** - Refer to the Drug Chemistry Section Technical Procedure for Drug Chemistry Analysis.

5.7 Calculations

5.7.1 Difference Calculation, round to two decimal places:
Standard Retention Time – Sample Analyte Retention Time = Difference in Time (+/-)

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

Agilent 6890 GC Instrument Manuals

Agilent 5973 and 5975 Instrument Manuals

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

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

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

9.0 Records

- GC-MS Logbook
- GC-MS Maintenance Log (see FORMS for printable version)
- GC-MS Activity Log (see FORMS for printable version)
- Case Record

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document Technical Procedure H-01 converted to ISO Standards.
10/26/2012	2	**5.3.1.2** Methandrostenolone and Testosterone Propionate shall be used for Monthly Performance check of HIGH temperature program. **5.3.1.3** Codeine/hydrocodone standard solution mixture shall be run on LOW temperature program. **5.3.1.8** Two steroids listed above shall be baseline resolved on HIGH program. Codeine/hydrocodone shall be baseline resolved on LOW temperature program. **5.7.1** Time Difference Calculation, round to two decimal places: Standard Retention Time – Sample Analyte Retention Time = Difference in Time (+/-) **5.3.2.1** and **5.6.2.4** corrected typos