	<p style="text-align: center;"><i>Instruments</i></p> <p style="text-align: center;">Pitt County Sheriff's Office Forensics Services Unit <i>Issued by the Technical Leader</i></p>	<p>Effective Date: 2018/10/22</p>	<p>Ver.: 3</p>
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1.0 Purpose - This procedure specifies the required elements for the calibration and use of the Agilent 7890B GC interfaced with the Agilent 5977B Series MSD.

2.0 Scope - This procedure applies to the GC-MS instrument used in the Drug Chemistry section of the Pitt County Sheriff's Office Forensic Services Unit.

3.0 Definitions

- **Performance verification** - The initial confirmation of the reliability of a previously or externally validated method or instrument.
- **Probability Based Matching** – 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.
- **Primary reference material** – Any reference material which has documentation issued by the provider authenticating its chemical composition.
- **Secondary reference material** – Any reference material used in the course of casework that has its chemical composition verified by a primary reference material.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Agilent Gas Chromatograph 7890B (GC)
- Agilent 5977B Series Mass Selective Detector (MSD)
- Agilent Automatic Liquid Sampler
- PC with Agilent Analytical Software, or equivalent
- Computer Printer or other data output device


4.2 Materials

- Sample vials and caps
- 10 µL syringe
- A non-polar capillary column with a (5%-Phenyl)-methylpolysiloxane stationary phase such as a DB-5MS or HP-5MS

4.3 Commercial Reagents

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

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4.4 Reference Materials

- Multi-component drug solutions
- Primary or Secondary reference material

5.0 Procedure

5.1 Performance Verification for New Instrument

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

5.1.2 The Technical Leader shall conduct the performance verification on new GC-MS instruments prior to use for casework. This shall include the following:

5.1.2.1 Successful tunes (See below) on three separate days.

5.1.2.2 The multi-component reference material standard solutions from the Monthly Performance Check (See below) shall be run 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 not be greater than 2.0 %.

5.1.2.3 The following shall be stored in Document Management (DM)

5.1.2.3.1 The manufacturer's serial number.

5.1.2.3.2 Date the performance verification was completed.

5.1.2.3.3 Daily tunes and data collected during the three day performance verification.


5.1.2.3.4 Any written documentation from the vendor after on-site installation.

5.1.3 A log shall be maintained near each instrument containing the following:

5.1.3.1 **Activity Log** shall include the date, sample identification, initials of operator, GC-MS method used, substances observed, and comments for each sample analyzed.

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


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

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- 5.1.3.2 Tune reports.** Tunes performed to check instrument performance during maintenance or troubleshooting need not be retained.
- 5.1.3.3 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.)
- 5.1.3.4 Monthly Performance Check** data shall be included if hard copies were printed. If the electronic option was used, the log shall contain the appropriate file name(s) on the dates the checks were run. Other retention time reference material data may also be stored in the log.
- 5.1.3.5** All information stored in the log shall be archived at least annually to an External Redundant Drive.

5.2 Maintenance

- 5.2.1** Record all maintenance in the log at the time it is performed.
- 5.2.1.1** The Technical Leader or designee shall update the instrument log when the instrument is ready to be placed back in service.
- 5.2.2** Record lengths of column trimmed in the log. If the column is trimmed, the instrument shall be out of service until a Monthly Performance Check is successfully completed (see below).
- 5.2.2.1** Standards run prior to the column maintenance shall not be used for retention time comparison after the column maintenance.
- 5.2.3 Routine maintenance** – The routine maintenance schedule is a suggested minimum guideline. The maintenance schedule will be determined by the Technical Leader 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 monthly when in use.
 - Post-maintenance check: Successful tune (See below).
- 5.2.3.3 Syringe**

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

5.2.3.4 Liner

- Replace as needed, or every six months.
- Post-maintenance check: Successful tune (See below)

5.2.3.5 Clean Source

- Clean annually, or when filaments are replaced.
- Post-maintenance check: Successful tune (See below) and Monthly Performance Check (See below).

5.2.3.6 Gold Seal

- Replace annually.
- Post-maintenance check: Successful tune (See below)

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 Technical Leader 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 below) shall be performed before the instrument is placed back in service.

5.2.5 Shutdown

5.2.5.1 A successful tune (See below) 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 Naming and Saving of Casework and Performance Check Files *("D" folders and all files contained therein)*

5.3.1.1 Files associated with casework and performance checks shall not be deleted or overwritten.

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- 5.3.1.2** Files shall be saved on the instrument computer hard drive according to the year/month in which they were collected. The files in the monthly folders shall be archived at least annually on the Pitt County Sheriff's Office external hard drive attached to the instrument.


5.3.2 Monthly Performance Check

- 5.3.2.1** 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. If the standard solutions are not run during the first seven days of the month, the instrument shall be out of service until the standard solutions are successfully run.
- 5.3.2.2** Two multi-component standard solutions made up of a variety of drugs commonly encountered in the laboratory shall be run.
- 5.3.2.3** 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.
- 5.3.2.4** 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 Technical Leader or designee.
- 5.3.2.5** 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 Technical Leader or designee.
- 5.3.2.6** The total ion chromatograms for each standard solution shall be visually inspected for resolution between the required components. Any deficiencies shall be documented in the instrument log and the instrument shall be evaluated by the Technical Leader or designee.
- 5.3.2.7** The chemist reviewing the monthly standard solution injections shall enter the retention time of each required component into the retention time comparison log. The reviewing chemist shall mark the activity log to indicate the successful runs of the standard solutions.

5.3.3 Blank injections

- 5.3.3.1** Prior to the injection of a sample, a blank solvent injection shall be made using the same method and split ratio as the sample.
- 5.3.3.2** The solvent shall be prepared by the individual chemist and be the same solvent from the same bottle used in the sample preparation.

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5.3.3.3 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 Technical Leader or designee.

5.3.4 Syringe flush

5.3.4.1 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.

5.3.4.2 Methanol shall be used in the first wash vial.

5.3.4.3 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. The instrument will not be tuned on days not in use. 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 A tune will be performed according to specifications listed in **Appendix A**. Perform the Standard Spectra Tune (stune) with Perfluorotributylamine (PFTBA) as the tuning standard.

5.4.3 Compare the tune report to previous tunes and notify the Technical Leader or designee of any major variations.

5.4.4 Record daily and post maintenance/shutdown tunes in the activity log along with initials, date, and a notation if the tune evaluation was acceptable for casework. Note any parameters that were out of specification before the acceptable tune was obtained.


5.4.5 Initial the tune report(s) and mark any parameters that are out of specification. File the tune report(s) in the log.

5.5 Instrument Procedure

5.5.1 If an instrument problem or error message occurs, the chemist who discovers the problem shall document the problem in the activity log. If the problem cannot be immediately corrected, the chemist shall mark the activity log to show that the instrument is out of service, notify the Technical Leader, and notify all other chemists affected.

5.5.2 Sample Preparation

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5.5.2.1 Refer to the [Technical Procedure for Extractions and Separations](#).

5.5.2.2 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.

5.5.2.3 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.

5.5.2.4 Derivatizing agents may be used when needed.

5.5.3 GC-MS Methods

5.5.3.1 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](#).

5.5.3.1.1 In the event a new GC-MS method needs to be developed refer to the [Laboratory Procedure for Validation of Technical Procedures](#) and the Performance Verification section above.

5.5.3.2 Descriptions of specific method parameters are located in **Appendix B**.

5.5.3.2.1 When GC-MS is being used as a screening technique, the GC method chosen shall screen for a wide variety of controlled substances, from phenethylamines to high molecular weight compounds such as JWH compounds and steroids.

5.5.3.3 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.

5.5.4 Sequences

5.5.4.1 The current date shall be used when naming a sequence. Sequences need not be archived.

5.5.5 Identification


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

5.5.5.2 Evaluate the chromatogram and spectra for peaks of interest.

5.5.5 For sample runs NOT used for identification, the case file shall contain at least the following:

- Total Ion Chromatogram (TIC) for the corresponding blank.

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- Total Ion Chromatogram (TIC) for the sample.

5.5.5.4 For sample runs used for identification, the case file shall contain:

- Total Ion Chromatogram (TIC) for the corresponding blank.
- Total Ion Chromatogram (TIC) for the sample.
- Mass spectra and corresponding library search of peaks of interest.
- Expanded mass spectra of phenethylamines and other compounds as needed.
- The requirements are the same for any reference material standards used for identification, or retention time comparison.

Note: The retention times may be determined by using an integrator in the Agilent software, or may be determined as the elution time at which the mass spectra were collected.

5.5.5.5 Mass Spectral Identification

5.5.5.5.1 The sample 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 chemist in the identification but shall not be used as the sole basis of identification.

5.5.5.5.2 The mass spectrum must contain all of the major ions unique to the analyte. 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.


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

5.5.5.5.4 When methamphetamine or phentermine are confirmed utilizing GC-MS in conjunction with preliminary testing, the retention time of a single sample shall be compared to the retention time of the respective reference material.

5.5.5.6 Retention Time (RT) Identification

5.5.5.6.1 Retention time data shall be required for the following:

- No preliminary tests are available for the substance identified by GC-MS.

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- Sample size does not allow for additional testing, other than GC-MS.

5.5.5.6.2 When sample size allows, a second sample shall be analyzed for mass spectral and retention time comparison purposes.

5.5.5.6.3 The requirement for retention time identification shall be retention time which, when compared to a reference material standard, has a difference by 0.10 minute or less. The retention time may be determined by using an integrator in the Agilent software or may be determined as the elution time at which the mass spectrum was collected.

5.5.5.6.4 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.

5.5.6 Reporting - Refer to the [Technical Procedure for Drug Chemistry Analysis](#).

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.
- 6.3** The 500LOW Methods cannot separate the coelution of ethylone and alpha-Pyrrolidinopentiophenone (alpha-PVP). If these substances are encountered in combination in casework, other methods of isolation (such as derivatization) may need to be considered.

7.0 Safety

- 7.1** Handle syringes with care to avoid punctures.
- 7.2** Use extreme caution dismantling/installing/transporting compressed gas cylinders. Cylinders shall not be moved without the cylinder cap securely in place.
- 7.3** Gas Chromatograph and Mass Spectrometer may be extremely hot. Avoid touching hot areas and wear protective gloves while performing maintenance.

8.0 References

Agilent instrument manuals for applicable models.

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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

- GCMS Maintenance Log
- GCMS Activity Log
- GCMS Retention Time Comparison Log
- Case file

10.0 Attachments


- Appendix A – Standard Spectra Tune Parameters
- Appendix B – GC Method Parameters

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Appendix A

Standard Spectra Tune Parameters

1. The mass assignments of the three tuning masses 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 Technical Leader or designee. The instrument shall remain out of service until the problem is corrected.
2. 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 Technical Leader or designee. The instrument shall remain out of service until the problem is corrected.
3. 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 Technical Leader or designee. The instrument shall remain out of service until the problem is corrected.
4. 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 Technical Leader or designee. The instrument shall remain out of service until the problem is corrected.
5. The abundance of any peaks less than 69 amu shall not be greater than 10 % of the abundance of the base peak.
6. Peaks at 18, 28 or 32 amu are indicative of water, nitrogen and oxygen, respectively, and may indicate an air leak.
7. 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 log. If the problem persists, document the deviation on the tune and in the activity log and notify the Technical Leader or designee. The instrument shall remain out of service until the problem is corrected.

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Appendix B

GC Method Parameters

Column dimensions: 30 m X 0.25 mm X 0.25 µm

HIGH METHODS – These methods are used for compounds that elute after 13min. in the screen method, e.g. buprenorphine, LSD, some steroids and some synthetic cannabinoids. Method run time is 25 min. and the sample injection is 1µL. Scan range is 40-500 amu. The following are the specific methods used:

HIGH100 – 100 split, 1.00 minute initial time, 280 °C initial temperature, 10 °C/minute ramp, 300 °C final temperature, 22.00 minute final time, 25.00 minute total run time

HIGH20 – 20 split, 1.00 minute initial time, 280 °C initial temperature, 10 °C/minute ramp, 300 °C final temperature, 22.00 minute final time, 25.00 minute total run time

HIGH5 – 5 split, 1.00 minute initial time, 280 °C initial temperature, 10 °C/minute ramp, 300 °C final temperature, 22.00 minute final time, 25.00 minute total run time

HIGHSL – No split, 1.00 minute initial time, 280 °C initial temperature, 10 °C/minute ramp, 300 °C final temperature, 22.00 minute final time, 25.00 minute total run time

LOWX METHODS – These methods are used for typical drug samples (cocaine/amphetamines/most opiates). It is used for compounds that elute before 16min. in the screen method. Method run time is 16min. and the sample injection is 1µL. Scan range is 40-500 amu. The following are the specific methods used:

LOWX100 - 100 split, 1.50 minute initial time, 100 °C initial temperature, 30 °C/minute ramp, 300 °C final temperature, 7.83 minute final time, 16.00 minute total run time

LOWX20 – 20 split, 1.50 minute initial time, 100 °C initial temperature, 30 °C/minute ramp, 300 °C final temperature, 7.83 minute final time, 16.00 minute total run time

LOWX10 – 10 split, 1.50 minute initial time, 100 °C initial temperature, 30 °C/minute ramp, 300 °C final temperature, 7.83 minute final time, 16.00 minute total run time

LOWX5 – 5 split, 1.50 minute initial time, 100 °C initial temperature, 30 °C/minute ramp, 300 °C final temperature, 7.83 minute final time, 16.00 minute total run time

SCREEN METHODS – These methods shall be used to screen samples when a substance is NOT previously indicated. Method run time is 35min. and the sample injection is 1µL. Scan range is 40-500 amu. The following are the specific methods used:


SCRN100 - 100 split, 1.50 minute initial time, 100 °C initial temperature, 30 °C/minute ramp, 300 °C final temperature, 26.83 minute final time, 35.00 minute total run time

SCRN20 – 20 split, 1.50 minute initial time, 100 °C initial temperature, 30 °C/minute ramp, 300 °C final temperature, 26.83 minute final time, 35.00 minute total run time

SCRN5 – 5 split, 1.50 minute initial time, 100 °C initial temperature, 30 °C/minute ramp, 300 °C final temperature, 26.83 minute final time, 35.00 minute total run time

500LOW METHODS – (To be used on an as needed basis) These methods are used to improve resolution between structurally similar compounds including but not limited to synthetic cannabinoids and phenethylamines.

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Method run time is approximately 36 minutes and the sample injection is 1uL. Scan range is 40-500amu. The following are the specific methods used:

500LOW100 – 100 split, 2.00 minute initial time, 70 °C initial temperature, 15 °C / minute ramp, 275 °C final temperature, 20.00 minute final time, 35.67 minute total run time.

500LOW20 – 20 split, 2.00 minute initial time, 70 °C initial temperature, 15 °C / minute ramp, 275 °C final temperature, 20.00 minute final time, 35.67 minute total run time.

500LOW5 – 5 split, 2.00 minute initial time, 70 °C initial temperature, 15 °C / minute ramp, 275 °C final temperature, 20.00 minute final time, 35.67 minute total run time.

500LOWSL – No split, 2.00 minute initial time, 70 °C initial temperature, 15 °C / minute ramp, 275 °C final temperature, 20.00 minute final time, 35.67 minute total run time.

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REVISION HISTORY		
CURRENT VERSION	EFFECTIVE DATE	SUMMARY OF CHANGES
1	2017/11/14	Original Document.
2	2018/04/01	<p>Entire document -Replaced “Illicit Drugs” with “Drug Chemistry” section, replaced “logbook” with “log”</p> <p>Title – Added “Drug Chemistry”</p> <p>Purpose – removed old instrument reference</p> <p>Original 5.2.3.5 – Removed “Pump Oil” section – This task no longer applies.</p> <p>Appendix B – Corrected total run time of 500LOW method</p>
3	2018/10/22	<p>Definitions – Updated primary reference material.</p> <p>Entire document – Removed references to run log.</p> <p>5.3.2.7 – Removed the requirement of printing (or printing to PDF) the total ion chromatogram of each standard and blank. Added requirement of reviewing chemist to enter the retention time of each required component into the retention time comparison log.</p> <p>5.4.4 – Clarified to record the daily and post maintenance/shutdown tunes in the activity log.</p> <p>9.0 – Added GCMS Retention Time Comparison Log</p> <p>Appendix B – Updated run times for 500LOW methods. Added LOWX methods.</p>