Name of Procedure:

Hewlett-Packard/Agilent 6890 GC interfaced to the Hewlett-Packard/Agilent 5973 and 5975 Series MSD/DS

Suggested Uses:

The gas chromatograph/quadrupole mass selective detector/data system is used to qualitatively identify compounds present in items of evidence.

The gas chromatograph/mass spectrometer separates mixtures of compounds and produces mass spectra of compounds. The mass spectrum and / or retention time of a compound can be compared to a standard for identification. If necessary, mass spectral libraries can be searched through computer-based matching software to aid in identifying unknown compounds.

Apparatus Used to Perform Procedure:

Hewlett-Packard/Agilent Gas Chromatograph 6890 (GC)
Hewlett-Packard/Agilent 5973 Series Mass Selective Detector (MSD) or
Hewlett-Packard/Agilent 5975 Series Mass Selective Detector (MSD)
Hewlett-Packard/Agilent Automatic Liquid Sampler
PC with Hewlett-Packard Analytical MSD Productivity ChemStation Software, or equivalent
Computer Printer or other data output device
Methanol
Hexane
Chloroform
Acetonitrile
Ethyl Acetate
Sample vials and caps
10*u*l syringe
DB-5 GC Column (or other appropriate column)
Helium Gas
Perfluorotributylamine [FC-43]

Calibration and Performance Verification of the Hewlett Packard 6890 GC/ 5973MSD and 6890 GC/5975MSD:

A. Calibration (Tuning)

1. The GC-MS is kept on at all times.
2. Calibration is performed each day that the instrument is in use with the Autotune program, using the Standard Spectra Tune option using Perfluorotributylamine (PFTBA) as a tuning standard.
3. Compare this Standard Spectra Tune file to previous ones and address any major variations which may indicate instrument problems.
   a. The three tuning masses in the upper profile part of the report should be within +/- 0.2 amu of 69.00, 219.00, and 502.00. Any deviation larger than +/- 0.2 amu indicates a problem.
   b. The peak widths of the three tuning masses should be 0.55 +/- 0.10 amu.
   c. The relative abundances should show that mass 69 is the base peak (100%).
      1. The relative abundance of mass 219 to mass 69 should be within 40 - 85%.
      2. The relative abundance of mass 502 to mass 69 should be within 2.0 - 5%.
   d. The isotopic ratios should be within the following ranges:
      1. For 70/69 the ratio should be within 0.5 – 1.6
      2. For 220/219 the ratio should be within 3.2 – 5.4
      3. For 503/502 the ratio should be within 7.9 – 12.3
   e. An air leak within the system may be indicated if the mass-to-charge ratio (m/z) at 28 is greater than m/z 18, or if either of these m/z ratios are greater than 10%.
      1) If an air leak is detected, the air leak should be isolated and corrected and the instrument retuned.
5. If any of the above stated parameters are out of specification, document the deviation in the activity log and file the tune printout in the tune section of the logbook. If a problem is identified, correct the problem and tune the instrument again. If there is no readily apparent problem or the problem persists, notify the GC/MS Coordinator.
6. The Chemist reviewing the tune will initial the printout and place it in the instrument notebook.

B. Performance Verification

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 will be placed Out of Service until the Standard solutions are successfully run.

a. A multi-component standard solution including codeine and hydrocodone will be run on the HIGH temperature program.
b. A multi-component standard solution including phentermine and methamphetamine will be run on the LOW temperature program.
c. Additional standard solutions may be run on a monthly basis to establish retention times. Any additional monthly standard solutions are not required to verify instrument performance.
d. Standard solution data files should be named with the first letter of each word of the standard solution name, followed by any numerical designation in the solution name, followed by the numerical month designation. The corresponding blank should have a similar name with a designation that it is a blank. Example: The name of an “Amphetamines Mix” standard solution run in Janurary would be “AM01” and the blank would be “AM01-b”.
e. If maintenance is performed that may affect retention times or the performance of the mass spectrometer, the standard solutions will be run before the instrument is placed back in service.
f. 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 will be documented in the instrument log and the instrument evaluated by the GC/MS Coordinator, ASAC or SAC prior to being used for casework.
g. The mass spectrum of each required component in the standard solutions will be compared to a reference spectrum. Any appreciable differences will be noted in the instrument log and the instrument evaluated by the GC/MS Coordinator, ASAC or SAC prior to being used for casework.
h. The total ion chromatograms for each standard solution will be visually inspected for sufficient resolution between the required components. For the high temperature program, codeine and hydrocodone should be baseline resolved. For the low temperature program, phentermine and methamphetamine should be resolved at a minimum of half-height. Any deficiencies will be documented in the instrument log and the instrument will be evaluated by the GC/MS Coordinator, ASAC or SAC prior to being used for casework.

i. The Chemist reviewing the required monthly standard solution injections will 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 will be initialed by the reviewing Chemist and placed in the "GC RT" section of the instrument notebook. The Chemist will also mark the activity log to indicate the successful run of the standard solution.

Application of Procedure:

These procedures do not cover every aspect of the instrument. The operator of the instrument should consult the manual(s) for the instrument.

A. Sample Preparation (suggested)

1. Powders and residues: Filter with appropriate solvent to prevent particulate matter and undesired compounds from being introduced onto the column (example: sugars). Derivatizing agents may be used when needed.
2. Tablets:
   a. Alprazolam, Lorazepam, Diazepam, etc.: Add several drops of solvent to an intact (uncrushed) tablet(s). Allow the tablet to soak for a short time. Transfer the solvent through a filter to a sample vial or insert and add more solvent for analysis.
   b. Coated tablets: Remove coating before adding several drops of solvent to the remaining intact tablet(s), prepare as described above. Pharmaceutical tablets should be extracted to remove large amounts of Acetaminophen or Aspirin prior to running on the GC/MS.
   c. Sulfates need to be extracted/converted before they can be introduced into the instrument.
3. Syringes: Wash with methanol and extract if necessary. (If excessive quantities of blood or other liquids are present in a syringe then an extraction is required).

B. GC/MS Methods:

1. The following are standard GC/MS methods used in the Drug Chemistry Section:
   a. **HIGH**
   2 minutes initial time, 120^{o}C initial temperature, 15^{o}C/minute, 275^{o}C final

temperature, 15 minutes final time, 27.33 minutes total time. Scan range = 45 – 450 amu.
Used for cocaine, opiates, benzodiazepines and general use.

b. **LOW**
2 minutes initial time, 70^{o}C initial temperature, 15^{o}C/minute, 275^{o}C final temperature, 15 minutes final time, 27.67 minutes total time. Scan range = 45 – 450 amu.
Used for phenethylamines and other volatile compounds.

c. **HVYHI** (Heavy High)
2 minutes initial time, 120^{o}C initial temperature, 15^{o}C/minute, 275^{o}C final temperature, 70 minutes final time, 82.33 minutes total time. Scan range = 45 – 500 amu.
Used for steroids, buprenorphine and other late eluting and/or higher molecular weight compounds.

2. Each of these methods may be used at split ratios of 5:1, 20:1, or 100:1. Numbers or an abbreviation in front of the method name indicates the split ratio
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.
4. When the standard methods are not sufficient to analyze a compound, a modified method may be used in accordance with the laboratory Quality Assurance Manual.

C. Sample Injection:

1. Prior to the injection of a sample, a “blank” solvent injection shall be made.
   a. The solvent shall be prepared by the individual chemist at the time of sample preparation and be the same solvent from the same bottle used in the sample preparation.
   b. The blank solvent injection will be evaluated to ensure that the instrument and solvent are free of any compound being identified in the sample and any compounds that may interfere with identification of the sample component(s).
2. The temperature program for the “blank” solvent injection will be the same one used for the injection of the corresponding sample. The split ratio should be the same for both injections.
3. The syringe must be flushed at least 10 times with clean solvent between injections to ensure the sample integrity between injections and that no sample

transfer is made between sample vials. Methanol will be used in the first wash solvent bottle and hexane or chloroform in the second solvent wash bottle.

D. Data:

After the data system has collected the data, examine the chromatogram and spectra for the peaks of interest, print or electronically transfer the following for the case file:

1. Total Ion Chromatogram (TIC) for the corresponding blank.
2. The Total Ion Chromatogram (TIC) for the sample.
3. The mass spectrum of peaks of interest.
4. The expanded mass spectrum of phenethylamines.

E. Reporting:

The GC/MS provides retention time data and mass spectral data.

1. The requirement for identification using the mass spectrum is a reasonable comparison of a standard and the sample's mass spectra.
   a. If the standard is from a Mass Spectral Library then only the mass spectrum with the library identified is included in the case file. Library search results may be included.
   b. If the standard is from a printed source then the mass spectrum and sufficient reference information to identify the source is included in the case file.
   c. If the standard is from a primary or secondary standard that has been run on the instrument then the following will be included:
      1. Total Ion Chromatogram for corresponding standard blank, including data file name.
      2. Total Ion Chromatogram for the standard, including data file name.
      3. Mass spectrum of standard and any other peaks of interest.
      4. Drug Chemistry Vault ID or supplier / lot number or appropriate Drug Chemistry designation.
2. The requirement for identification using the retention time is a retention time match between the standard and sample within + / - 2.0 %. The retention time may be determined by using an integrator in the Chemstation software, provided that the same integrator and parameters are used for the standard and sample. The retention time may also be determined by the time at which the mass spectrum was collected.

a. The standard may be run within thirty days before or after the case file sample if no column maintenance has been performed between time of injection of the standard and sample that would change the retention times. If the 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.
b. The standard data must be included in the case file and consist of:
    a. Total Ion Chromatogram for corresponding standard blank, including data file name.
    b. Total Ion Chromatogram for the standard, including data file name. If an integrator is used then the retention times must be displayed.
    c. Mass spectrum of the standard and any other peaks of interest. If an integrator is not used then the mass spectrum must display the time that it was collected.
    d. Drug Chemistry Vault ID or supplier / lot number or appropriate Drug Chemistry designation.

F. Instrument Logbook:

A logbook consisting of four sections will be maintained near each instrument.

1. The Activity "Log" section will document injections made on the instrument beside the appropriate date. The log will include the date, sample identification, initials of operator, GC/MS method used, and comments. Septum changes and any unusual error messages or the like should be recorded in this section. This will assist the GC/MS Coordinator when diagnosing instrument problems. **Use the attached Activity Log form to document injections and other data listed above.**
2. If samples are rerun for any reason, a new entry will be placed in the logbook. (Solvent runs used for maintenance purposes need not be recorded.)
3. The "Tune" section will contain the daily tune reports. Tunes performed to check instrument performance during maintenance or troubleshooting do not need to be retained.
4. The "Maintenance" section will document the date, descriptions of work performed, parts replaced, and the initials of the person performing or documenting the maintenance. (Septum changes do not need to be logged in the maintenance section. These entries can be recorded in the "Log" section as

outlined above.) **Use the attached Maintenance Log form to document maintenance performed.**

5. The "GC RT" section will contain monthly standard solution data. Other standard data may be retained in this section.
6. All instrument logbooks will be restarted with blank activity log forms on or about the first workday of the new calendar year.
7. Activity logs, Tunes, and GC Retention Data from each instrument notebook will be compiled yearly, labeled with instrument serial number, and placed in a packet near the instrument.
8. Data may be archived to the packet as the year progresses and the notebooks become full.

G. Sequence Files:

The current date can be used to name the first sequence of the workday. These sequences can be deleted after approximately one week and do not need to be archived.

H. Data Files:

1. Data file names should include the year designation and the case file number. This will ensure data from different years with the same file number will not be overwritten.
2. Data files associated with casework and standard solutions will not be deleted or overwritten. Data will be archived and labeled with the instrument serial number and dates as needed. Notify the GC/MS Coordinator if the disk drive(s) become full.

I. Responsibilities:

1. It is the Chemist's responsibility to ensure that the daily tune is performed and that the data is reviewed, printed, initialed and filed in the "Tune" section of the instrument logbook. A note will be placed on the activity "log" page if any problems occur to prevent starting new samples.
2. It will be the GC/MS Coordinator's responsibility to run the monthly Standard Solutions and document the status of the standard solution runs in the activity log. However, it is the Chemist's responsibility to ensure that all instrument

requirements have been met prior to running samples.

3. If an instrument problem occurs, the Chemist who discovers the problem will document the problem in the activity log and, when feasible, notify all other Chemists affected.

J. Instrument Problem Reporting:

1. Any problems related to instrument performance or error messages should be documented in the activity log.
2. Chemist's should notify the GC/MS Coordinator of any performance problems they cannot solve/correct.
3. Schedule requests for outside service through the GC/MS Coordinator, SAC or ASAC On-Call.

K. Suggested Maintenance Schedule

The maintenance schedule is only a suggested guideline. It does not address all maintenance. Actual maintenance times will be at the discretion of the GC/MS Coordinator and will depend upon instrument usage and performance.

1. Daily: Rinse and / or fill wash vials.
2. Weekly: Replace septum, inspect syringe.
3. Monthly: Replace injection liner.
4. Bi-annually: Change pump oil.
5. Annually: Clean source, replace gold seal.

Safety Concerns:

A. Avoid syringe punctures of hands and fingers.
B. Use extreme caution handling organic solvents to avoid contact with skin and eyes.
C. Use extreme caution dismantling/installing/transporting compressed gas cylinders.
D. Caution: Gas Chromatograph and Mass Spectrometer may be extremely hot.

Literature References:

Agilent 6890 GC Instrument Manuals

Hewlett-Packard 5973 and 5975 Instrument Manuals

Moffat,Jackson, Moss and Widdop, **"Clarke's Isolation and Identification of Drugs"**; 2nd Ed., Vol. 1, 1986.

Skoog,Holler, Nieman, **"Principles of Instrumental Analysis"**;5th Ed., 1998.

Maintenance Records for Agilent 597_ GC/MS # ____ Serial Number _____

Date	Initials	Maintenance Performed	Added to log?

Activity Log for Agilent 597_ GC/MS # ____ Serial Number _____

Date	Lab Number	Item #	Chemist	Method	Remarks