A. **PURPOSE**:

Gas Chromatography/Mass Spectrometry (GC/MS) is used in this laboratory to provide qualitative identification of analytes, and, when applicable, quantitative analyses. The GC/MS provides both retention time and fragmentation pattern information for analytes.. Comparison of data (samples versus positive controls) provides the basis for qualitative identification. For this procedure, GC/MS data is tentative identified using library spectra for comparison. Confirmatory identification is achieved by comparison of both chromatographic and mass spectral data. Although the GC/MS is a single instrument, it is considered two (2) separate analytical techniques.

B. **RESPONSIBILITY**:

All analysts within the unit who are authorized to perform casework in the Drug Chemistry discipline.

C. **DEFINITIONS**:

GC/MS: Gas Chromatography/Mass Spectrometry

Instrument Check Solution: a substance put into every GC/MS sample that demonstrates the instrument had an acceptable injection and is working as expected. This is generally CHEP in methanol but may be a similar solution if needed per case requirements.

D. **REAGENTS**:

1. Chloroform (reagent grade or equivalent)
2. Cyproheptadine (CHEP) (reagent grade or equivalent)
3. CHEP/MeOH (0.001% ; 10ppm): ~10mg of CHEP in ~1000mL of methanol (Instrument check solution ; need not be quantitatively accurate)
4. CHEP/EtOAc, (0.001%, 10ppm): ~10mg of CHEP in ~1000mL of ethyl acetate (Instrument check solution ; need not be quantitatively accurate)
5. Ethyl Acetate (reagent grade or equivalent)
6. Methanol (reagent grade or equivalent)
7. Various glassware and materials (variable suppliers)
8. Daily Standard: Secobarbital, Oxycodone, PCP, Cocaine, delta-9-THC, Heroin - in CHEP solution. Made from DEA exempt certified standards. This is a mixture of substances commonly found in samples. The composition may change based on the needs of the unit.
9. Combo Control: amphetamine and trazadone standards in CHEP solution.

E. **PROCEDURE**:

1. GC/MS SET UP:

   a. GC/MS Daily check: Prior to an instrument being used for casework on any given day an air/water check, tune, and daily standard solution are performed or analyzed. (Note: If the instrument is still running a sequence from the night [previous day], instrument evaluation can be done at the conclusion of the sequence and no interruption is necessary simply because the days have changed). A check list is utilized for documentation and to demonstrate that the instrument is 'Acceptable for Use.' The checklist is filed in the maintenance log book for each instrument. (See CS-7.2)

   b. In the event of a failure of one of the set-up parameters (air/water check, tune, or daily standard), the analyst will work to determine the cause of the failure. This may include the need for basic maintenance. If the problem is corrected the check sheet is marked as acceptable for use and any troubleshooting or solutions should be documented. If the problem still occurs and the instrument should not be used, it will be marked appropriately as not acceptable for use. Analysts will work with the Lead Examiner to determine the issue with the out-of-service instrument and try to correct the issue.

   c. All appropriate printouts (e.g., air/water checks, tunes and the documentation of any maintenance performed) is located in the maintenance log book for each instrument. The printout of the daily standard is kept in a file within the instrumentation room.

      i. Auto tunes/Quick Tunes:

         (a) Generally auto tunes are run the first day of each month, quick tunes on each other day of operation.

         (b) Tune Parameter Review:

            (i) Correct mass assignments (69, 219 and 502)

            (ii) Peak widths (acceptable range 0.45-0.65)

            (iii) Relative ion abundances (69 at 100%, 219 >30% and 502 >4%)

            (iv) Isotope masses (1 AMU +/- 0.2 of parent peak)

            (v) Isotope ratios: 70 (0.5%-1.5%), 219 (2%-8%), 503 (5%-15%)

            (vi) EM voltages are recorded to help track the condition of the source.

      ii. Air and Water Checks:

         These are performed each day of use to verify that there are no leaks in the GC system. The ions corresponding to water (m/z 18), nitrogen (m/z 28) and carbon

dioxide (m/z 44) are checked to verify values are less than 10% of m/z 69 and to track any trend that may suggest a leak.

iii. Daily Standard Mix (Qualitative)

(a) This can consist of PCP, Secobarbital, Cocaine, CHEP, THC Oxycodone, Heroin and other appropriate drugs. This mix is analyzed each day to verify that there are no unexplainable shifts in analyte retention times and that mass spectra are consistent. The printout of the daily standard is maintained in a file in the instrumentation room. The Daily Standard Mix analytes may be altered based on the requirements of the Unit.

(b) When preparing the stock Daily Standard Mix record the volumes used and lot numbers on the Daily Standard Stock Solution Reagent Log sheet (See CS-7.3). The log sheet is maintained in a log book within the Unit.

(i) The date made will be the lot number assigned to the Daily Standard Mix solution. This date must be on any aliquots of the stock solution. A date put in use must be documented on form CS-7.3.

(ii) Since this mixture will be assessed each day of use no expiration date will be assigned to the mixture. If it is determined that the stock solution is no longer acceptable (generally due to contamination or compound breakdown) the analyst will destroy the stock solution and any aliquots of the substance.

(c) The following parameters are reviewed and documented on the GC/MS Daily Maintenance Log (CS7.2)

(i) Target Peaks are present and Non-target peaks (if present must be at an acceptable level or explainable, such as the presence of a 6-MAM peak due to the breakdown of Heroin)

(ii) Retention time of CHEP (verify that no major unexplainable shifts have occurred)

(iii) Ratio of Abundance of Heroin to CHEP acceptable: indicating no major loss of sensitivity seen (e.g., 20% decrease from previous day).

(iv) Heroin spectral match is acceptable as compared to a published reference spectra.

d. Instrument Maintenance:

i. The analysts will use their knowledge and experience to determine if maintenance is required for an instrument. No maintenance should be performed on an instrument without notification to the Lead Examiner.

ii. Septa: Analysts will evaluate if septa need to be changed based on the results of instrument evaluation.

iii. Liners – Analysts will evaluate if septa need to be changed based on the results of instrument evaluation. (Note: Decreased sensitivity for heroin and excess "unexpected" peaks indicate the need to change a liner.)

iv. Liner seals: Analysts will evaluate if liner seals need to be changed based on the results of instrument evaluation.

v. Clipping column/Changing columns: Analysts will evaluate if column maintenance is needed based on the results of instrument evaluation. Loss of sensitivity or the presence of "unexpected" peaks may indicate the need for column clipping or changing.

vi. Switching/changing filaments: Loss of current or complete/partial signal loss indicates a possible filament change.

vii. Cleaning the source: An increase in electron-multiplier voltage values may indicate that the source needs to be cleaned.

viii. Other: as required due to instrument function and based on the results of instrument evaluation.

e. Instrument Checks: Most samples prepared for GC/MS injection contain CHEP (Cyproheptadine) in methanol or in an appropriate solvent. Samples (blank, control, sample, or standard) are placed in CHEP and act as a performance check for each injection into the instrument.

(i) Blanks: To verify that there is no carryover in-between case samples.

1. As the first sample in a set of samples
2. Between different sample types in one case
3. At a minimum of every five (5) injections if multiple like items from a single case are run
4. Between different cases

(ii) Additional Instrument Check Solution: CHEP is used to show the instrument is working properly as determined by the expected retention time and mass

spectral fragmentation pattern. The addition of CHEP to samples allows analysts to determine if valid injections were made. (Note: CHEP can be made in solvents other than methanol if analytes of interest are not soluble in methanol or if methanol causes artifacts to be formed (e.g., designer amphetamines or derivatizations)).

2. Sample Preparation:

Samples for analysis by this methodology can range from powder, rock, residue, plant material or tablet. Samples extracted or otherwise prepared can be handled as a residue once the extraction process is completed. This includes, but is not limited to, plant material, tablets, liquids and food stuffs. (See SOP CS-4).

a. Sample log: samples which are to be run by GC/MS are listed on a GC/MS Sample Position Worksheet (see CS-7.1). This is a map of sample position on the auto sampler. It is acceptable for GC/MS vials to only be labeled with the vial position (this is considered properly labeled), since this map will allow the identity of the sample to be tracked. The sequence printout can also be used to track the sample vials as long as the printouts are checked and documented.

b. Powder or Rock Samples:

i. Add approximately 1ml of CHEP/MeOH (or CHEP/EtOAc, as appropriate) to an appropriately labeled GC/MS vial, using an automatic transfer pipette.

ii. Add ~1 mg sample to the vial, using a new applicator stick.

iii. Cap the vial, reserve for analysis.

iv. Prepare CHEP blanks and controls as appropriate for the run. Due to the nature of sample set-up for powder and rock samples CHEP is the reagent blank or negative control for the run.

c. Residue samples (including samples extracted or otherwise prepared so that they are now in residue form)

Note: because of the variable nature of residue samples, analyst discretion will be employed in this process. Unusual or exceptional cases should be discussed with the Lead Examiner or Deputy Director to ensure method applicability.

i. To the dried residue, previously prepared in a GC/MS vial, add 1ml of CHEP in MeOH (or CHEP in EtOAc, as appropriate) and cap the GC/MS vial.

(a) Note, because of the potential for variable sample concentration, a dilution may be necessary to adjust concentration of the solution (e.g., CHEP) to facilitate the analysis. Controls will be treated in the same manner as the sample whenever possible.

ii. Prepare CHEP blanks, controls and reagent blanks as appropriate for the run.

d. Tablets: tablet samples can be run direct or as a residue if an extraction method was used.

i. If running direct prepare as a Powder/Rock sample listed above. If running after extraction, run as a residue sample listed above.

ii. Prepare CHEP blanks, controls and reagent blanks as appropriate for the run.

iii. Manufactured tablets with an identifiable imprint will be analyzed by searching for drug information using the logo and reputable literature, whether hardcopy or online). Information obtained will be documented in the casefile along with references. Such evidence may additionally be analyzed by other techniques (e.g., FTIR, GC/MS) for confirmation purposes, if necessary. Evidentiary tablet analyses can be stopped after information is obtained through a logo search only if tablets are not suspected to be illicitly produced and upon approval from either the Lead Examiner or Deputy Director. Subsequent reports shall convey the appropriate limitations to a logo-only search.

3. GC/MS Analysis:

a. Each GC/MS is checked and evaluated on each day of use, prior to the analysis of any samples (Note: If instrument is still running from night before, evaluation can be done at conclusion of initial run). This process is documented in an instrument specific maintenance log book Once an instrument is marked as acceptable for use it can then be considered acceptable for the day's case work. (see CS-7.2)

b. It is acceptable to have changes to the temperature programs as needed for specific cases as long as the appropriateness of the change is demonstrated by properly working blanks and controls. Any variations beyond a simple temperature hold in the temperature method will be documented in the case file, either by a notation or a printout of the temperature method.

c. Samples prepared as above should be injected using the GC/MS method "Drugs.m" or "COMBO.m" or other method as appropriate, (See below– method details, column, temperatures, etc.).

i. The Drugs.m method is appropriate for most samples in this section. This includes plant extractions, most tablets, rock and powder samples. This method is not appropriate for most steroids since they are very late eluting substances and some of the designer methamphetamine and amphetamines since they are low molecular weight substances which require a gentler ramping program.

ii. The Combo.m method is a combined method that encompasses both early and late eluting drugs, this is generally used for powder, tablet and rock samples that are negative by the Drugs.m method. This method is a long method that will detect early eluting substances such as amphetamines and late eluting compounds such as trazadone.

When using this method a Combo control should be run to demonstrate that the full range of drugs would be found.

iii. Copies of methods are maintained with each instrument so that any time the exact instrument parameters can be identified for a sample. If a new method is developed a copy will also be maintained.

iv. When there is an indication of the type of substance present (such as through a tablet imprint) the standards books can be consulted to see which temperature program is appropriate for the compound of interest.

d. Sequence: The sequence of samples, blanks and controls is written on the "GC/MS Sample Position Worksheet" (CS-7.1) or kept on the sequence entry log section in the software; this is to act as a map of the auto sampler. This is kept on a clip board next to the specific instrument, periodically these are removed from the clipboard and filed in a file within the instrumentation room.

i. The sequence of samples is typed into the GC/MS Instrument Vial position, method program, sample identification and instrument operator are entered. The exact sequence of controls, reagent blanks, CHEP blanks and samples will vary with each run, general guidelines are as follows;

(a) Negative control

(b) Positive control

(c) CHEP blank

(d) Samples (no more than five like samples are to be run without a blank being injected)

CHEP blanks or negative controls must be run:
1) In-between different cases, or
2) In-between differing sample matrixes within a single case, or
3) In-between like sample matrixes if packaged in separate evidence bags.
Variation from this can occur if directed by the Lead Examiner or Deputy Director.

(e) CHEP blank

(f) Reference standards as needed.

(i) Note: "Daily Standard Mix" is acceptable as the reference standard for all analytes contained in the mix. When using this data, the printout must contain a comparison to a published reference standard.

1. Example: If the case contains cocaine, the Daily Standard will be printed with the TIC, the Cocaine spectra and the cocaine reference spectra.

(ii) Standards are run for the comparison of the RT and spectra (known to unknown).

1. It is preferable to have the standards run within 24 hours of the original injection however if not possible the analyst must assure that there has been no work done to the instrument that would affect the results, such as clipping the column would affect the RT.

(g) If running the COMBO method, the Combo Control must be injected this is only required once per day on the instrument used.

4. Interpretation of Data:

a. The TIC and Spectra for each sample is reviewed under the Enhanced Data Analysis Screen of the GC/MS. Each peak is reviewed to determine if it is that of a controlled substance.

i. Preliminary identification of an analyte is achieved by either operator recognition of a particular spectral profile, or by the instrumental library providing matching spectral information.

b. An identification is considered "confirmed" when the sample analyte is compared to an in-house validated standard; and the 3 following criteria are demonstrated:

i. Relative Retention time consistent (+/- 5%) between sample and validated standard.

(a) Note: exceptions may be made for this based on the substance in question, for example a wider window of acceptance may be accepted for steroids or non-controlled substances that do not chromatograph well (e.g., stanozol and acetaminophen).

ii. Spectrum of sample consistent with Instrumental Library.

(a) Reference libraries including, but not limited to, Wiley, AAFS and SWGDRUG) and an internal library. The internal library is made of substances run by the laboratory that are not in the published libraries. Entries in the internal library should be compared to a published spectra from a reliable source such as the manufacturer, Micrograms or other journal.

iii. Spectrum of sample consistent with validated standard, this will have been run in concordance with the unknown samples.(See section D (f) (ii) above)

(a) Note: in rare cases a substance may be identified by 'mass spectral match only' when an in-house standard is not available, but a published reference spectra is used for the id. This must be clear in the final report that the identification is by

mass spectral match only. This will generally only be used for non-controlled substances where GC/MS Spectra is consistent to a published reference.

c. "Cuts" or non-controlled substances alone or mixed with controlled substances: most street samples have non-controlled items mixed with them. When a substance is preliminarily identified, a standard of the substance is run.

Non-controlled substances mixed with controlled substances need not be identified, however the following guidelines should be considered when determining if the cut should be identified:

(a) "Cut" peaks greater than the peak of interest may be identified and reported it is deemed important to the case.

(b) For substances which are found to contain only a non-controlled substance (caffeine, lidocaine, acetaminophen etc.) the substance identified will be reported, the requirements for reporting are the same as for controlled substances.

5. Acceptance of Data:

a. To accept GC/MS analyses the following must be true:

i. The blank prior to the injection of the sample must not contain significant unexplainable peaks. It is always preferable to have clean blanks however due the nature of samples run in the section (such as plant materials or oil based samples) certain non-controlled substances will elute after multiple injections.

(a) If the analyte being identified in the sample is present in the blank prior to its injection, the abundance of the peak must be less than 5% of the CHEP peak.

(b) When it is known that there is a carry-over issue with a sample, multiple blanks can be run in-between injections to minimize the carryover.

ii. Blanks run after a sample injection should be free of any analytes of interest. Any analytes found in the blanks indicate that the temperature program chosen was not long enough to elute all the peaks from the GC column.

iii. The controls must be acceptable.

(a) Negative controls, as well as blanks being analyzed prior to evidentiary samples, will be free of analytes-of-interest and no significant unexpected peaks should be present. Explainable peaks will be acceptable.

(b) The positive control must contain at least the analyte-of-interest for an identification to occur.

(c) Unexpected results within positive or negative control data will be reported to the Lead Examiner for evaluation.

iv. Where appropriate, the chromatograms and mass spectra of evidentiary samples will be compared to mass spectral libraries and in-house standards in order to identify any substances.

v. When an analyte identified is one which is found in the daily standard mix or in the combo control, and it is analyzed contemporaneously with the same method, the component within the daily standard mix can be used as the positive control.

b. Documentation: Appropriate instrumental printouts including reagent blanks, controls, standards and samples will be included in the case file, such that an independent reviewer would be able to readily understand the basis for the analyst's conclusions.

i. The TIC (Total Ion Chromatogram) will be retained in casefiles. Label the chromatographic peaks for all analytes being identified and the CHEP peak and indicate if the retention time is acceptable (e.g., *RT OK or RT* √).

(a) Every significant peak will be evaluated. Indications that peaks have been searched will be done either with a check mark or other method.

(b) Analysts may choose to label non-reported/non-confirmed analytes, such as by labeling the peak and putting the label in parenthesis or making some other notation indicating it is not confirmed.

ii. All identified analytes will have documented spectra and appropriate comparative reference spectra (e.g., from spectral libraries) within the casefiles.

iii. In samples where no analytes are identified the analyst should include the TIC along with any appropriate extracted ion chromatograms (EICs). If there are significant peaks that cannot be identified, or attributed to common artifacts, the spectra should be included in the case.

iv. In cases where identifications cannot be made due to the lack of obtaining a positive control, indications can be made based on mass spectral library data, if appropriate.

v. Positive controls will be analyzed contemporaneously when identifying analytes in evidentiary samples or when specifically looking for analytes in samples which result in negative results.

GC/MS Common Temperature Programs (may be changed if needed):

Temperature program	Drugs			Combo		
Initial temp	90° C			60° C		
Initial Time	0.00 min			0.00 min		
Ramps	rate	temp	Final time	rate	temp	time
Rate/final	30.0	300	7.50	20	200	0.0
				30	300	7.0
Post temp	0°c			280°c		
Post time	0.00			0		
Run Time	14.5 min			6.83 min		
Front inlet						
Mode	Pulsed splitless			Pulsed splitless		
Initial temp	250°c			250°c		
Pressure	9.32psi			8.19 psi		
Pulse pressure	40.0psi			40.0psi		
Pulse time	0.50 min			0.50 min		
Split Ratio	n/a			n/a		
Split Flow	n/a			n/a		
Total flow	48.1mL/min			53.4 ml/min		
Gas type	Helium			Helium		
Injection volume	1 microliter			1 microliter		
Post injection washes	Solvent A -3 Solvent B - 3			Solvent A -3 Solvent B - 3		
Tune file	ATUNE			ATUNE		
Acquisition mode	Scan			SIM		
Solvent delay	4.95 minutes			3.73 min		
	Low mass	High Mass	Threshold	Low mass	High Mass	Threshold
	44	550	500	44	550	500
	MS Quad 150°c max 200°c			MS Quad 150°c max 200°c		

A record of instrument temperature programs will be maintained within the laboratory. When updates to these programs are made the temperature program will be printed and maintained within a "Temperature Program" log book. The start date of use will be written on the printout and the analyst will initial the document.

F. **REFERENCES**:

1. Clark's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids, and Post-Mortem Materials, The Pharmaceutical Society of Great Britain.
2. The Drug Identification Bible

Revision #	Revision History
3	Made slight grammatical and format modifications. Added a 'Revised History' section to the document. Updated logo searching part of the procedure. Strike-outs removed and color uniformly set as black. Replaced 'section' with 'unit.' Replaced section supervisor with Lead Examiner. Summarized steps so that they were not wordy. Replaced Laboratory with Unit. Corrected CHEP error from 1% to 0.001% concentration. Removed the option that the Daily Standard Mix solution's concentrations can be altered based on analyst's decision. Added that no maintenance should be done on instruments without Lead Examiner notification.