A. **PURPOSE**:

Gas Chromatography/Mass Spectrometry (GCMS) is used in this laboratory to provide both initial, qualitative identification of analytes, as well as quantitative analysis, where applicable. The GCMS provides both retention time, and fragmentation pattern information for analytes, parameters which are dependent upon different chemical characteristics. Comparison of such information with reference data, and verified standard materials, provides the basis for qualitative identification with an extremely high degree of confidence. For this procedure, GCMS data is generally collected in "Scan" mode, and tentative qualitative analyte identification made based on spectral information. Confirmatory identification is achieved by comparison of both spectral and retention time information to validated standard materials, co-analyzed with samples.

B. **RESPONSIBILITY**:

All analysts (however titled) assigned to the CS section are responsible to follow the guidance of this SOP when utilizing a GC/MS for case analysis.

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 (Mallinkrodt, "Nanograde," or equivalent)
2. Cyproheptadine (CHEP; Aldrich or equivalent)
3. CHEP/MeOH, 1%: ~10mg of CHEP in ~1000ml of methanol (Instrument check solution; need not be quantitatively accurate)
4. CHEP/EtOAc, 1%: ~10mg of CHEP in ~1000ml of ethyl acetate (Instrument check solution; need not be quantitatively accurate) Note; Varying concentrations of CHEP can be utilized to facilitate analysis of special cases.
5. Ethyl Acetate; (Mallinkrodt, "Nanograde," or equivalent)
6. Methanol (Mallinkrodt, "Nanograde," or equivalent)
7. Validated Standard materials as appropriate (Variable Suppliers)
8. Daily Standard: Secobarbital, Oxycodone, PCP, Cocaine, delta-9-THC, Heroin in CHEP. Made from DEA exempt certified standards. This is a mixture of substances commonly found in samples, the makeup can change and analytes added as trends change.
9. Combo Control: amphetamine and trazadone standards in CHEP.

10. Adulterant Mix: a mix of standards of non-controlled substances commonly used as "cuts" in street drugs, the make up of this mix will change as cut popularity changes.

E. **PROCEDURE**:

1. GC/MS SET UP:

a. GCMS Daily check: Prior to an instrument being used for case work on any given day an air/water check, tune and daily standard are run. (Note: If instrument is still running from night before, evaluation can be done at conclusion of initial run). A check list is utilized to document this and to demonstrate that the instrument is 'Acceptable for Use', this 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, if not it is marked as not acceptable for use. If an instrument is marked as not acceptable for use the analyst will work with the section Supervisor to determine the issue and correct the issue. The printouts of the air/water checks, tunes and the documentation of maintenance performed is located in the Maintenance log book for each instrument. The print out of the daily standard is kept in a file within the instrumentation room.

i. Auto tunes/Quick Tunes:

(a) Note; 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 (1AMU +/- 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.

1. Other tune programs, such as the STUNE can be run if needed for special projects.

ii. Air and Water Checks;

(a) 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 then 10% of m/z 69 and to track any trend that may suggest a leak.

iii. Daily Standard Mix; (a qualitative mixture)

(a) This consists of PCP, Secobarbital, Cocaine, CHEP, THC and Heroin. This mix is run at the start of each day to verify that there are no unexplainable shifts in the retention times and that the fragmentation patterns are consistent. The print out of the daily standard is maintained in a file in the instrumentation room. The Mix components may be altered based on the requirements of the Laboratory.

(b) When preparing the stock Daily Standard Mix record the volumes used and lot number on the Daily Standard Stock Solution Reagent Log sheet (See CS-7.3). The log sheet is maintained in a log book within the CS laboratory. Since column changes and instrument conditions can affect the concentration needed of the individual components of the Daily Standard the preparer should refer to the volumes of components used for the last preparation. Experience and knowledge of the working condition of the instruments will allow the preparer to determine if the volume of a single component should be increased or decreased.

(i) The date made will be the lot number assigned to the Daily Standard mixture. 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.

c. Instrument Maintenance:

i. The analysts will use their knowledge and experience to determine if maintenance is required for an instrument.

ii. Septa –analysts will evaluate if the septa needs to be changed based on the daily instrument set up, (air leak, extra :unexpected" peaks in the daily standard)

iii. Liners – analysts will evaluate daily standards to determine if liners need to be changed. Decreased sensitivity for heroin and excess "unexpected" peaks indicate the need to change a liner.

iv. Liner seals – analysts will use their knowledge and experience to determine if the linear requires changing

v. Clipping column/Changing columns – analysts will use their knowledge and experience to determine if a column needs clipping or changing. Loss of sensitivity, or the presence or "unexpected" peaks may indicate the need for column clipping or changing.

vi. Switching/changing filaments- indicated by a loss of current

vii. Cleaning the source – indicated by increased EMVOLTS

viii. Other – as required due to instrument function

d. Instrument Checks: Each sample prepared for GC/MS injection contains CHEP in methanol or appropriate solvent.

i. Instrument Check Sample: every sample (blank, control, sample or standard) is placed in CHEP (Cyproheptadine in methanol or appropriate solvent). The CHEP acts as a performance check for each injection into the instrument.

(i) Blank, CHEP is run as a blank to verify that there is no carryover between case samples. This is run minimally:

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 injections if multiple like items from a single case are run
4. Between different cases

(ii) Instrument check solution, CHEP is used to show the instrument is working properly as determined by the expected retention time and fragmentation pattern.

1. The addition of CHEP to every sample allows the analysts to determine if a valid injection was made, since the CHEP peak with appropriate RT, ion abundance and fragmentation pattern will be seen.

a. Note: CHEP can be made in a solvent other than methanol if the analyte of interest is not soluble in methanol, or if methanol causes artifacts to be formed with the compound (such as can be seen with some of the designer amphetamines).

2. <u>Sample Preparation</u>:

Samples for analysis by this methodology can range from powder, rock, residue, plant material or tablet. Samples extracted or other wise 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, considerable analyst discretion will be employed in this process. Unusual or exceptional cases should be discussed with the Section Supervisor, QA Manager, 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. Note: manufactured tablets with an identifiable imprint need not be analyzed by GC/MS as long as tablets are not suspected as being illicitly produced.

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 'MSTOP/Enhanced program', Table Log. 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) Reagent blank (or negative control)

(b) Positive control

(c) CHEP blank

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

(i) Note: CHEP blanks or negative controls must be run between different cases or between differing sample matrixes within a single case or between like sample matrixes if packaged in separate evidence bags. Variation from this can occur if directed by the section supervisor or ~~Laboratory~~ 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 such as stanozol and acetaminophen.

ii. Spectrum of sample consistent with Instrumental Library.

(a) Reference Libraries include published reference libraries (including PMW Tox, 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 should be discussed with the section Supervisor prior to reporting and it 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 ~~tablets in which there is a preliminary identification by manufactures imprint code (such as by the Drug Identification Bible, PDR or on-line source) and the~~ 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. An Adulterant mix standard is available for the identification of many cuts, this consists of a number of popularly seen cuts and the make-up of this may vary as new cuts become popular. When a substance is preliminarily identified that is not in the adulterant mix 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 then the peak of interest may be identified and reported if it is deemed important to the case.

(b) ~~"Cut" peaks that are baseline (less then 10%) of the peak of interest need not be identified.~~

(c) ~~"Cut" peaks that are significant >10% should be identified. Identification can be preliminary and need not reported but noted in the case~~.

(d) 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 a GC/MS run 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) If fatty acids or other non-controlled substances are present, as is often seen due to "dirty" samples being injected they must be explained.

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

ii. Blanks run after a sample injection should be reviewed to assure that there are no peaks of interest that eluted from the sample before. This would indicate that the temperature program chosen was not long enough to elute all the peaks from the prior sample.

(a) Example: a sample containing trazadone run under the drugs method will appear negative, the blank after the sample injection will contain a trazadone peak. This would indicate that the sample needs to be run under the COMBO method.

iii. The controls must be acceptable.

(a) The blank control will be clean, with no significant unexpected peaks present. Note that certain peaks may be present if they are a product of the extraction performed.

(b) The positive control must contain the analyte of interest (such as caffeine in a plant material extraction) other peaks may be present if explainable.

(i) If an unexpected controlled substance is found in either the positive or negative control, work may need to be done to determine if the analyte was introduced in the laboratory. The section supervisor will be consulted to determine what action needs to take place.

(ii) In rare cases if the positive control is run too weak (so the drug of interest is not seen) but the negative control is acceptable and the samples are positive the Supervisor or Deputy Director can approve the run. A notation will be added to the case file to annotate why the run is acceptable, this will be initialed and dated by the Supervisor or Deputy Director.

iv. Unknown peaks will be compared to reference library standards and in-house standards to identify any controlled substances.

(a) When the substance identified is a substance that is found in the daily standard mix or in the combo control and they are run in the same method the control can be used as the standard; no additional standard need be run. The data sheet must be printed in a manner to document traceability to a reference spectra. The TIC, drug spectra with a spectra of the library match

(i) Example: any unknown run in the DRUGS method and found to contain heroin, marihuana, cocaine, PCP, secobarbital, or any other analyte that has been added

to the mix, can be compared to the daily standard run as part of the instrument set up that day.

b. Documentation: Original, or copies of appropriate instrumental print-outs 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) is reviewed; Label the 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) Analysts are not required to label each peak on a TIC but they are required to search all the peaks and to indicate that they searched them 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. The spectra for the peaks of all reported analytes will be included. For each analyte reported a minimum of one reference library spectra will be included in the case file.

      (a) Example: a case with multiple samples each with cocaine being reported; each sample cocaine spectra will be included, one will be compared to a reference library spectra of cocaine.

      (b) In samples were no substance is identified the analyst will include the TIC for the run. If there are significant peaks that cannot be identified (other than those that are clearly fatty acids or hydrocarbons) the spectra should be included once in the case.

         (i) In cases where the identification cannot be formally made such as the lack of a standard this will be noted on the GC/MS printout.

   iii. The TIC, Spectra, and reference library spectra for any standards run to identify an unknown will be included in the case file. Note that in some cases it may be important to include standards of drugs that are not identified in the case. Lot number or other identifier must be recorded either on the GC/MS data sheet or in the case file, this will allow for traceability.

      (a) Example: a case submitted believed to contain LSD however no drugs are identified by GC/MS. The TIC and spectra for the LSD standard run under the same temperature program as the sample is included in the case file to demonstrate that LSD would be identified under the instrument conditions.

   iv. If there are unacceptable runs, such as samples run too strong so that they are repeated after dilution the analyst may choose to include only the TIC of those runs with an explanation of why they are being repeated. The spectra for the run need not be included, since they are not being used to make the conclusion.

      (a) Example: Run to strong, repeating after dilution.

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

GC/MS Common Temperature Programs:

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		

Note: these parameters are general guidelines for the methods there will be differences between the various instruments in the section. Changes may be made due to instrument maintenance such as clipping the column.

A record of instrument temperature programs will be maintained within the laboratory section; when updates to these basic programs are made (due to column changes or other reasons) the temperature program will be printed and maintained with in a “Temperature Program” log book. The start date of use will be written on the printout and the analyst will initial the document.