A. PURPOSE:

Quantitations are generally performed in the Controlled Substance laboratory for any of the following case types:

Drug diversion cases where the concentration of the substance is needed to demonstrate whether drugs are being diverted from patients.

Federal cases where they need to determine the weight of the pure drug, most commonly this is performed for liquid PCP cases.

Any State or Local case where a criteria weight for cocaine or heroin is met or exceeded and there is an indication (through normal qualitative analysis) that the drug may only be present in trace amounts

When necessary, a case were an overdose is being investigated and it is suspected that "high" concentration of drugs may be present

Uncertainty will be considered and reported for all drug diversion cases, and whenever the results can be used to influence a sentence. In general cocaine and heroin quantitations will not require the uncertainty to be reported; Connecticut state law requires only that the drug be present in the aggregate item not at any specific concentrations.

B. SAFETY:

Proper PPE will be worn when handling case materials and other substances as appropriate. This will include at minimum a laboratory coat and disposable gloves. MSDS sheets should be consulted for standards if there are any questions about proper PPE.

C. RESPONSIBILITY:

a. All analysts (however titled) assigned to the CS section are responsible to follow the guidance of this SOP when performing quantitations.

b. Section Supervisor: is responsible to assure uncertainty is determined and documentation is maintained within the section.

D. DEFINITIONS:

a. SIM – Single Ion Monitoring. A method on the GC/MS which can be set to monitor specific ions

b. SCAN – method on the GC/MS which monitors a range of ions

c. CHEP – Cyproheptadine

d. IS – Internal Standard

E. PROCEDURE:

1. When performing quantitations, analysts must assure that the method chosen meets the needs of the customer/sample. Example: A method that has a quantitation limit of 0.5 mg/ml would not be acceptable for a diversion case where the sample is suspected to be in a concentration of 0.1 mg/ml.

2. Lot numbers of any reference standards used in a quantitation will be recorded on the GC/MS data sheets, or be recorded in the case file.

3. Drug Diversion Quantitations: drug diversion cases can vary widely and it is impossible to address all the possible quantitations that could be performed in the CS laboratory. The following are general guidelines for the performance of drug diversion quantitations by GC/MS:

    a. A one-point calibration is used with two controls. The calibrator and controls are made by two separate analysts (one making the calibrator and the other making the two controls). Whenever possible certified reference standards will be used to make the calibrator and controls. When possible different lots will be used. The standards will generally be made up in the same solvent as the certified reference material. If powdered standards are used they must be validated by UV to determine the exact concentration (see SOP CS-9).

    b. In general a 0.5 ~~1~~mg/ml solution is used as the calibrator

    c. Controls will generally be ~ 1 mg/ml and 0.25 mg/ml, bracketing the control.

d. The analyte of interest must be considered when determining if this range is acceptable (example: low dose drugs may require a lower range to be covered).

e. Controls will be considered acceptable if they are +/- 20% of the expected range.

f. Whenever possible an exemplar is obtained for diversion cases, this is very important for solutions made in viscous matrices. When an exemplar is submitted it will be prepared in the same manner as the samples but treated as a control for the method.

    i. For exemplar controls it is expected that the value will be +/-20% of the theoretical value.

g. All quantitations will be performed using an internal standard; this will be CHEP, unless CHEP proves to be an inappropriate IS for the analyte in question.

h. It is preferable to set up the abundance of IS and calibrators so that when injected into the GC/MS, the response of the IS is ~ equal to the response of the analyte of interest.

i. The IS is generally introduced in the last stage of sample preparation, the IS will be added in a predetermined amount.

j. The same amount of IS is added to all samples, the calibrator, and the control.

k. The GC/MS can be run in SIM or SCAN mode to perform the quantitation.

    i. For SIM mode quantitations it is desirable to pick three ions for the analyte of interest and two for the IS to base the quantitation on.

l. Samples will be diluted so that they are in the range of the calibration.

    i. Sample response should be no higher than the control and no lower than the lowest control (+/-5%).

m. If a single sample is submitted for analysis the sample will be prepared and run in duplicate. If the submission includes multiple like items the section Supervisor will be consulted to determine the number of items to be analyzed.

n. In most diversion cases there is usually a single item submitted. If there are multiple items, the analyst and supervisor will need to consider the question being asked. (Example: Is there a large scale manufacturer's issue, with medicines being produced in the wrong concentration, or is it a instance were they are trying to discover is an individual is diverting the substance).

o. Data collected by the GC/MS should be presented in an appropriate report.

i. Data represented in the quant report automatically calculates the values based on the calibrator. This data is transferred to an EXCEL spreadsheet to account for the dilutions made (see CS-12.1) this spreadsheet is an example of what can be used. The form can change slightly as long as all the needed information is present.

ii. Data represented in a SCAN report format will be transferred to an EXCEL spreadsheet to determine the sample concentrations (see CS-12.2). This spreadsheet is an example of what can be used. The form can change slightly as long as all the needed information is present.

iii. Once the "batch" sheet is prepared, the analyst submits the batch sheet with the GC/MS paperwork to a second analyst for review. This review will include:

1. Review of paperwork to assure there are no transcription errors

2. Review of controls to assure that they are within acceptable range

3. Review of dilutions to assure that they are accounted for and are logical.

4. Example: a 1:100 dilution is reported, the reviewer needs to check the math for the dilution.

iv. When reporting a quantitation on a drug diversion case, the uncertainty associated with the determination of concentration will be reported. (See SOP CS-2 and the Uncertainty budgets).

4. Quantitation of Street Drugs: Some quantitated substances in the CS laboratory are Cocaine, Heroin, and PCP (liquid form). PCP is quantitated for federal cases where they need to determine if the liquid contains >10 grams of pure PCP. The penalty incurred can be affected by these results therefore uncertainty will be determined and reported.

    a. Cases that may warrant a quantitation include large single bags of powder where the GC/MS response during normal qualitative identification indicates that the drug may only be present in trace amounts. Analysts must use their training, experience and knowledge to aid them in this determination.

5. The information provided in this method for GC/MS temperature programs (below) are guidelines for the program parameters. Each instrument in the section will have different parameters depending on the column type, length, and other factors unique to the instrument.

6. Cocaine:

    a. Sampling: The Laboratory will provide quantitative analysis based on the customer's needs.

    b. Examples of sample Preparation (see CS-5.1):

        i. ~15-25 mg of the substance is weighed into a scintillation vial using the balance designated for quantitation work. Record the weight on the batch worksheet (CS-12.1).

            1. Note based on the analyst's knowledge of the case the analyst can use their knowledge, experience and skills to judge the proper amount of sample used for the initial dilution

                a. Example: for a CSF with a lot of cut the analyst may choose to use more sample for the initial sample dilution.

        ii. 10 ml of methanol is added to the vial

        iii. The vial is capped and swirled to mix

            1. Note: for smaller quantities the analyst may chose to homogenize the materials prior to weighing out the portions for quantitation.

This cannot be done for large samples, especially cocaine salt form due to the likely hood of causing wide scale laboratory contamination.

c. Sequence set up: each of the calibrator, controls, and standards is pipetted into a labeled GC/MS vial, mixed, and then transferred into a micro vial.

i. Controls: a certified reference material (CRM) will be used whenever possible to make the calibrator and controls. The lot number of the CRM is recorded on the quant batch sheet (CS-12.1) The controls are diluted and made as follows (other levels can be used as needed for the sample):

ii. Calibrator: 100 ul IS + 50 ul standard+ 50 ul Methanol
Control 1: 100 ul IS + 100 ul standard
Control 2: 100 ul IS + 25 ul standard + 75 ul Methanol

iii. The value of the calibrator and controls will be based on the certified value supplied by the manufacturer.

iv. Blanks: IS only

v. Samples: 100 ul of the mixed sample dilution and 100 ul of IS.

vi. The sequence in the GC/MS will be a blank, the calibrator, blank, high control, low control, blank, samples.

vii. A blank will be run between every set of samples.

viii. If additional samples are added to the initial batch (by the same or another analyst) they must run a control, a blank, and then the samples.

d. GC/MS:

i. The sequence is entered in as normal with the method COCQUANT being chosen as the method (See CS-12.4)

ii. The samples are run per the method

iii. Open the Enhanced Data section and process the data.

iv. The calibration is updated to the calibrator run that day.

v. The controls and samples are compared to the calibrator using the reporting feature.

vi. Print all the quantitation reports

e. Compiling Batch: the analyst compiles the batch which includes printing all the GC/MS reports and completing the batch sheet.

i. Using the Quantitation Batch Summary Sheet in EXCEL fill in the needed information

ii. The EXCEL spreadsheet will calculate the % of cocaine in each sample.

iii. Print the spreadsheet.

iv. Once completed the analyst either accepts or refuses the batch (based on batch acceptance below) by circling Y or N under batch acceptable. If the batch is not acceptable an explanation must be added to the batch sheet stating why it is unacceptable.

f. Batch Acceptance:

i. Controls: the high and low control must be within +/- 20% of the expected value

ii. Blanks should be clean; this will be defined as no peak at the retention time of cocaine that is more than 3% of the IS.

iii. Samples:

1. the 2 portions of the same item or 2 “like” items; should not vary more not more than 20%

2. the response of the cocaine must be within the range of the low control and calibrator.

3. For all samples, controls and the calibrator the ions must be within the set ranges.

g. Batch Review: a second analyst (someone other than the one that prepared the Cocaine Quantitation Batch Sheet) will perform a review of the batch including:

   i. Review the items listed under batch acceptance assure they are acceptable

   ii. Review the GC/MS printouts and compare the data to the batch sheets, check for transcription errors.

   iii. Initial each page of the batch.

   iv. The reviewer determines if the batch is acceptable and initials and dates the batch sheet.

   v. If a batch is not acceptable the reviewer initials and dates the batch sheet and writes an explanation for why it is not acceptable.

      1. If there were unacceptable quant batches, a copy of the batch sheet is placed in the case file, with the complete batch being filed in the CS laboratory files.

h. Batch Paperwork: the batch paperwork is split up so that for each case associated with the batch a copy of the batch sheet and the GC/MS printouts related to that case are filed in the specific case file. The original batch sheet and the GC/MS data for the calibrator, controls, and blanks are filed in the CS laboratory files.

   i. If there were unacceptable quantitation batches a copy of the batch sheet is placed in the case file, with the complete batch being filed in the CS laboratory files.

i. Reporting: the final value is based on the form of the drug identified, if the sample is free base the concentration is reported as the free base amount if it is salt form it is reported as the salt form concentration.

   i. Example:

1. Item 1A1 was determined to contain 68.7% cocaine as free base
2. Item 1A1 was determined to contain 68.7% cocaine as salt form (e.g.HCl)

7. Heroin:

a. Sampling: The Laboratory will provide quantitative analysis based on the customer's needs (see CS-5.1):

b. Sample Preparation:

i. ~10-20 mg of the substance is weighed into a labeled scintillation vial using the balance designated for quantitation work. Record the weight on the batch worksheet (CS-12.2). A witness will be present while the case is opened and unsealed for sample preparation.

1. Note: Based on the analyst's knowledge of the case the analyst can use their experience to judge the proper amount of sample used for the initial dilution
2. Example: For a heroin sample with a lot of cut the analyst may choose to use more sample for the initial sample dilution.

ii. 5 ml of methanol or appropriate solvent is added to the vial

iii. The vial is capped and swirled to mix

1. Note: For heroin pellets, a portion (or two portions if required) of the pellet can be isolated and homogenized prior to making the initial dilution(s).

c. Sequence set up: each of the calibrator, controls, and standards is pipetted into a labeled GC/MS vial, mixed, and then transferred into a micro vial.

i. Controls: A certified reference material (CRM) is used whenever possible to make the calibrator and controls. The lot number of the CRM is recorded on the quant batch sheet (CS-12.2) The controls are diluted made as follows:

ii. Calibrator: 100 ul IS + 50 ul standard+ 50 ul Methanol or other solvent
Control 1: 100 ul IS + 100 ul standard
Control 2: 100 ul IS + 25 ul standard + 75 ul Methanol or other solvent

iii. The value of the calibrator and controls will be based on the certified value supplied by the manufacturer.

iv. Blanks: IS only

v. Samples: 100 ul of the mixed sample dilution and 100 ul of IS.

vi. The sequence in the GC/MS will be a blank, the calibrator, blank, high control, low control, blank, and samples.

vii. A blank will be run between every set of samples.

viii. If additional samples are added to the initial batch (by the same or another analyst) they must run a control, a blank, and then the samples.

d. GC/MS:

i. The sequence is entered in as normal with the appropriate method being chosen as the method (See CS-12.4)

ii. The samples are run per the method

iii. Open the Enhanced Data section and process the data.

iv. The calibration is updated to the calibrator run that day.

v. The controls and samples are compared to the calibrator using the reporting feature.

vi. Print all the quant reports

e. Compiling Batch: The analyst compiles the batch which includes printing all the GC/MS reports and completing the batch sheet.

i. Using the Quantitation Batch Summary Sheet in EXCEL fill in the needed information

ii. The EXCEL spreadsheet will calculate the % of heroin in each sample.

iii. Print the spreadsheet.

iv. Once completed the analyst either accepts or refuses the batch (based on batch acceptance below) by circling Y or N under batch acceptable. If the batch is not acceptable an explanation must be added to the batch sheet stating why it is unacceptable.

v. Both acceptable and not acceptable batches are then given to a second analyst for review.

vi. Batch Acceptance:

vii. Controls: the high and low control must be within +/- 20% of the expected value

viii. Blanks should be clean; this will be defined as no peak at the retention time of heroin that is more than 3% of the IS.

ix. Samples:

1. The Two portions of the same item or Two "like" items; should not vary more not more than 20%

2. The response of the heroin must be within the range of the low control and calibrator.

3. For all samples, controls and the calibrator the ions must be within the set ranges.

x. Batch Review: a second analyst (someone other than the one that prepared the Heroin Quantitation Batch Sheet) will perform a review of the batch including:

1. Review the items listed under batch acceptance assure they are acceptable.
2. Review the GC/MS printouts and compare the data to the batch sheets, check for transcription errors.
3. Initial each page of the batch.
4. The reviewer determines if the batch is acceptable and initials and dates the batch sheet.
5. If a batch is not acceptable the reviewer initials and dates the batch sheet and writes an explanation for why it is not acceptable.
6. Batch Paperwork: the batch paperwork is split up so that for each case associated with the batch a copy of the batch sheet and the GC/MS printouts related to that case are filed in the specific case file. The original batch sheet and the GC/MS data for the calibrator, controls, and blanks are filed in the CS laboratory files.
7. If there were unacceptable quant batches, a copy of the batch sheet is placed in the case file, with the complete batch being filed in the CS laboratory files.

xi. Reporting: the final value is based on the salt form (hydrochloride) of the drug.

1. Example: Item 1A1 was determined to contain 34.2% heroin as salt form (e.g. HCl).

8. Phencyclidine (PCP): The Laboratory will provide quantitative analysis of Liquid PCP based on the customer's needs (see SOP CS-5.1). For these cases the quantitation is based on the volume of sample present. Uncertainty will be determined and reported for PCP quantitations unless a reason is noted in the case file.

    a. PCP quantitations are run on a SCAN or SIM method on the GC/MS. The concentration is determined based on the response of the PCP to that of the internal standard.

b. Sampling: In general limited numbers of items are submitted for analysis with this type of case. All items submitted will be quantitated (in duplicate) unless there is a reason not to (note reason in case jacket). In the rare case that large numbers of items are submitted consult with the section supervisor.

   i. If a single item is submitted two portions of the sample will be analyzed.

c. Sample Preparation:

   i. A dilution suitable to the sample is prepared by taking X ul of the sample and diluting it in ethyl acetate or appropriate solvent. In general liquid PCP is strong; the analyst should review the last PCP quantitation batches to see the general trend of the samples being submitted as a starting point for the initial dilutions. A witness will be present while the case is opened and unsealed for sample preparation.

   ii. Sequence set up: each of the calibrator, controls, and standards is pipetted into a labeled GC/MS vial, mixed, and then transferred into a micro vial.

   iii. Controls: a certified reference material (CRM) is used whenever possible to make the calibrator and controls. The lot number of the CRM is recorded on the quant batch sheet (CS-12.3) The controls are diluted in ethyl acetate or appropriate solvent as follows:

      1. Calibrator: 100µL PCP standard diluted to 10 mls
      2. High Control: 200µL PCP standard diluted 10 mls
      3. Low Control: 50µL PCP standard diluted to 10 mls
      4. The value of the calibrator and controls will be based on the certified value supplied by the manufacturer.
      5. Blanks: IS only
      6. The calibrator, controls, and samples are then prepared as follows in labeled GC/MS vials: 100 ul of the respective solution and 100 ul of IS.

7. The sequence in the GC/MS will be a blank, the calibrator, blank, high control, low control, blank, samples.

8. A blank will be run between every set of samples.

d. GC/MS:

   i. The sequence is entered in as normal with the appropriate method being chosen as the method (See CS-12.4).

   ii. This method can be a SIM or SCAN program.

   iii. The samples are run per the method

   iv. Open the Enhanced Data section and process the data.

   v. Print all the following for each the blanks, calibrators, controls, and samples: the TIC, spectra of PCP and CHEP and the integration report.

e. Compiling Batch: the analyst compiles the batch, which includes printing all the GC/MS reports and completing the batch sheet.

   i. Using the Quantitation Batch Summary Sheet in EXCEL fill in the needed information

   ii. The EXCEL spreadsheet will calculate the % of PCP in each sample.

   iii. Print the spreadsheet.

   iv. Once completed the analyst either accepts or refuses the batch (based on batch acceptance below) by circling Y or N under batch acceptable. If the batch is not acceptable an explanation must be added to the batch sheet stating why it is unacceptable.

   v. Acceptable batches are then given to a second analyst for review. Not acceptable batch sheet copies are kept with the case file

   vi. Controls: the high and low control must be within +/- 20% of the expected value

vii. Blanks should be clean; this will be defined as no peak at the retention time of PCP that is more than 3% of the IS.

viii. Samples:

1. The Two portions of the same item or Two "like" items; should not vary more not more than 20%

2. The response of the PCP must be within the range of the high and low control.

3. For all samples, controls and the calibrator the peak shape should be acceptable (not split or misshapen)

ix. Batch Review: a second analyst (someone other than the one that prepared the PCP Quantitation Batch Sheet) will perform a review of the batch including:

1. Review the items listed under batch acceptance assure they are acceptable

2. Review the GC/MS printouts and compare the data to the batch sheets, check for transcription errors.

3. Initial each page of the batch.

4. The reviewer determines if the batch is acceptable and initials and dates the batch sheet.

5. If a batch is not acceptable the reviewer initials and dates the batch sheet and writes an explanation for why it is not acceptable.

6. Batch Paperwork: the batch paperwork is split up so that for each case associated with the batch a copy of the batch sheet and the GC/MS printouts related to that case are filed in the specific case file. The original batch sheet and the GC/MS data for the calibrator, controls, and blanks are filed in the CS laboratory files.

7. If there were unacceptable quant batches a copy of the batch sheet is placed in the case file, with the complete batch being filed in the CS laboratory files.

8. Reporting: the final value is based on the concentration of the pure drug.

    a. Example: Item 1A1 was determined to contain 34.2% Phencyclidine.

9. SOURCES OF ERROR:

    a. Not updating EXCEL fields required for calculating concentrations of analytes

    b. Improper dilutions, not recording the correct value of sample taken for quantitation

    c. Pipette Errors – malfunction or improper use

    d. Not updating GC/MS quant parameters (such as retention time) after clipping the column

    e. Not updating software with the correct calibrator concentration