History for the
GC/MS Quantitation SOP

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Approval

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Deleted appropriate
Proper sections
Validation

Standard Operating Procedures
For the Quantification of Solid Dosage Drugs
Using GC/MS with Internal Standards

1.0.0 Background

Under normal circumstances quantification of a substance's purity is not part of the analytical scheme used by the Idaho State Police Forensic laboratories. By special request this analysis can be performed. Typically this analysis is performed on casework that will ultimately be tried in federal court.

2.0.0 Scope

Although the following procedures have only been tested using cocaine and methamphetamine, the principals behind them are sound and will allow for the analysis of other controlled substances as long as the appropriate analytical standards, solvents and internal standards are used.

3.0.0 Equipment and Reagents

3.1.0 Gas Chromatograph/ Mass Spectrometer (GC/MS) and corresponding software.
3.2.0 Standards of the analyte of interest.
3.3.0 Appropriate GC or pesticide grade solvent. Chloroform for cocaine and methamphetamine. Use chloroform for mixing with the internal standards as well.
3.4.0 Volumetric flasks (10 and 25ml).
3.5.0 Gas tight syringes in a variety of sizes. (2.5ml, 250ul, and 25ul work well)
3.6.0 Internal standards: n-Tridecane for the phenethylamines and n-Octacosane for cocaine.

4.0.0 Generation of Standard Curve

A linear five-point calibration curve with a correlation coefficient of 0.995, or better, is required. In order to reduce the number of samples that need to be diluted the concentrations of the standards used to generate the curve should span the widest possible range at while maintaining linearity.

4.1.0 Accurately prepare a stock solution of your standard at 10,000 ug/ml. Make a solution of the internal standard also at 10,000 ug/ml.
4.2.0 Using the gastight syringes, sample vials, and serial dilutions prepare at least six standards of various concentrations, for example 5000, 2500, 1000, 500, 250, and 100ug/ml. Using at least six levels allows one to be thrown out if it is an outlier instead of remaking another standard.
4.1.0 Place 1.0 ml of each standard in an autosampler vial and add 100ul of the internal standard. **NOTE**: all additions to the vial should be injected through the septa in order to minimize vaporization of solvent.
4.3.0 Using the GC/MS software set up the calibration acquisition parameters and tables. For Hewlett Packard/Agilent Chemstation software the parameters and

tables are found in the data analysis/ calibration section.

5.0.0 Sample Preparation

One of the basic requirements in determining an accurate quantification is that the sample must be homogenous. The sample must also be prepared using the same extraction procedure that was used in generating the standard curve.

5.1.0 Place the entire sample into a mortar and grind with a pestle until a fine powder is formed. Depending on sample and mortal size numerous separate grindings may be required. Any separate grindings will be recombined and then thoroughly mixed.

5.2.0 Take a representative sample and weigh on an analytical balance, that is accurate to at least 0.1 milligram, and place into a volumetric flask (0.1 grams into a 10ml flask works well, 0.2g in 25mls is better.). Add solvent, shake to dissolve, and fill to the line.

5.3.0 Using the 2.5 ml syringe, remove 1.0mls of extract and place in an autosampler vial. Add 100ul of internal standard and analyze. **NOTE**: all additions to the vial should be injected through the septa in order to minimize vaporization of solvent.

5.4.0 Ideally the concentration of the sample extract should fall in around the midpoint of the calibration curve. If a sample's response is greater than the standard response of the highest point used in generating the curve then the sample must be diluted and reanalyzed.

6.0.0 Calculation of Final Results

Using the equation of the valid curve, calculate the concentration in the vial (the computer software should do this). Use the following equation to calculate the concentration of the analyte in the original sample:

$$\frac{(\mathbf{A}\ \text{ug/ml}) \times (\textbf{Milliliters of solvent}\ldots\text{include any dilution factors}) \times 100}{(1000) \times (\mathbf{B}\ \text{mg})} = \%\ \text{analyte}$$

A = Concentration given by curve
B = Weight of sample used, in milligrams

7.0.0 Notes

7.1.0 The curve must be linear

7.2.0 The area counts of the internal standard should be consistent from beginning to the end of the run (+/- 10% of the mean).

7.2.0 Because of the rarity of the requests for quantification it would be unusual to need to run samples more than twenty- four hours after the generation of the curve. If it is necessary to run samples past the twenty fourth hour, a midrange standard will be run and the resultant concentration will be within (+/-) 15 % of the known value. If it is not then the standard can be repeated. If it is still outside the 15% range then a new curve needs to be generated.

7.3.0 Injector should have a split liner with a glass wool plug.

7.4.0 It must be shown that each instrument and each analyst performing the analysis can generate reproducible results.

7.5.0 It is acceptable to use either manual or instrument generated integration. The analyst must be consistent however; the integration method used to generate the curve must be used with the corresponding sample set.

7.6.0 Each instrument that will be used to generate quantitative data will have to have it's own set of control limits.