#11
Analytical Method
For the Quantification of Methamphetamine
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. This analysis will only be performed on casework in which a federal court has a stated interest. This analytical method was derived from the principles and methods detailed in EPA publication "SW-846" and the states of Oregon and Utah's quantitation analytical methods.

2.0.0 Scope

2.1.0 The following procedures have only been approved for the analysis of samples containing methamphetamine in a solid matrix. The Idaho State Police Laboratory reserves the right to reject any samples for quantitative analysis based on sample size or circumstance.

2.2.0 In order to minimize the largest potential source of error, samples that have a high moisture or solvent content need special consideration.

2.3.0 At the laboratory's discretion all samples containing methamphetamine will be analyzed as a composite unless specifically requested by the prosecutor. Before compositing, each sample will be analyzed for the presence of methamphetamine.

3.0.0 Equipment and Reagents

3.0.0 Gas Chromatograph/ Mass Spectrometer (GC/MS) and corresponding software.

3.1.0 Solid methamphetamine hydrochloride. The purity is to be documented with a certificate of analysis from the vendor.

3.3.0 ACS grade chloroform stabilized with either ethanol or pentene.

3.4.0 Class A volumetric flasks.

3.5.0 1.0 ml Gastight® type syringes. Syringes that are used to generate the standard calibration curve will have their accuracy checked before each use via section 7.8.0 of this AM. The verification must encompass the expected working range of the syringe, 200ul and 800ul. Syringes that fail to meet the acceptance value of (+/-) 3% will be evaluated for accuracy and if necessary replaced. A syringe check is good for two weeks.

3.6.0 Internal standard. With a ratio of 1.3 ml of (98% or greater) n-tridecane per 1 L chloroform, prepare at least one liter. Each sequence of samples and standards must be made with the same internal standard.

3.7.0 0.5 N sodium carbonate solution. Add 2.7g of sodium carbonate to 100mls of water.

4.0.0 Generation of Standard Curve

A six point calibration curve will be generated.

4.1.0 Prepare a standard stock solution of approximately 2,000 ug/ml. Accurately weigh approximately 40-50 mg of methamphetamine, add to a 25ml volumetric flask and dissolve and bring to volume with the internal standard. Calculate the concentration.

4.2.0 Using the syringe, auto-sampler vials, and stock standard prepare an additional five 1.0 ml standards. Into five autosampler vials place 0.1, 0.2, 0.4, 0.6, 0.8 ml of stock std and then dilute to 1.0 ml using the internal standard. The undiluted stock standard must be one of the points on the curve. If the stock standard point does not fall within the linear range of the instrument then a more dilute stock standard is prepared and a new curve is run or the acquisition parameters of the instrument can be altered, i.e. split ratio, and the original curve rerun.

4.3.0 Add approximately 100 ul (3 drops) of a 0.5N sodium carbonate solution to each vial and mix.

4.4.0 Using the GC/MS software set up the calibration acquisition parameters and tables. The curve is to be generated using linear regression with the points weighted using the inverse square. 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. If a sample has been previously qualitatively analyzed and been resubmitted for quantitative analysis the sample must be reweighed before proceeding to 5.1.0. This new weight will be used in the final calculations. Contact with the discipline leader will be documented on samples that exhibit an abnormally high level of moisture or solvent.

5.1.0 Initially rough grind the sample with a mortar and pestle until the entire sample will pass through a US No. 4 sieve. Roll and quarter the sample until a representative sub sample of about 10 grams is obtained. Grind the sub sample until a fine powder is formed. **NOTE:** If the sample is less than 10 grams then grind the entire sample into a fine powder.

5.2.0 Using an analytical balance that is accurate to at least 0.1 milligram, accurately weigh out an amount of sample that is equal to, or less than, what was used for the stock standard, and place into a 25 ml volumetric flask. Add internal standard, dissolve, and bring to volume.

5.3.0 Into an auto sampler vial aliquot approximately one milliliter of sample extract, add approximately 100ul (3 drops) of 0.5 N Na_2CO_3, mix and analyze.

5.4.0 Samples are to be run in duplicate (two separate weighings and extractions). The results are averaged before being used for calculating the final result. The duplicate results must have a Relative Percent Difference (RPD) (labeled differential in the BEAST LIMS) of less than (+/-) 10%, if they are not then either first rerun the extracts or proceed to extracting a new pair of samples and analyze.

$$RPD = \frac{(R1-R2)}{} *100$$

A

Where R1 = Result of first run in percent
R2 = Result of second run in percent
A = Average of R1 and R2

5.5.0 If a sample(s) is to be forwarded to another laboratory for quantitative analysis, the originating laboratory will analyze the sample(s) qualitatively; prepare the sample(s) as per 5.1.0 above and then send a maximum of 1g per sample to the laboratory doing the quantitative analysis. If the original sample is less than 5 grams then the original sample can be sent without preparation. The submitting agency may also send the entire sample, without preparation, at their discretion.

5.5.1 The samples will be composited at the originating laboratory by mixing all of the samples that tested positive qualitatively for methamphetamine and the resultant mixture is then processed per section 5.1.0.

6.0.0 Calculation and Reporting of Final Results

6.1.0 Calculation

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. All calculations may be done by hand or by using computer software:

$$\frac{(\mathbf{A}\ \text{ug/ml}) \times (\mathbf{Milliliters\ of\ solvent}) = \mathbf{C}\ \%\ \text{analyte}}{(10) \times (\mathbf{B}\ \text{mg})}$$

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

If C is less than 20% then the sample is re-extracted and reanalyzed using a larger sample size. For calculating any re-extraction use the weight, in mg, of methamphetamine used to make the stock standard. The calculated result of the re-extraction must be greater than 20%.

6.2.1 Reporting

Using the formula:

$$\frac{\mathbf{C \times D} = \mathbf{X}}{\mathbf{100}}$$

Where **C=** average of the two duplicate results from the equation in 6.1.0
(if the result is greater than 100% the results will be calculated at 100%)
D= total weight of sample in grams

We will also report out the uncertainty range of each sample, by weight, using:

X x (+/-0.07) = Range

Report the result that "All samples calculated as the hydrochloride salt"

Each report will have the statement, "The expanded uncertainty value was calculated at the 95% confidence level.

6.2.2.1 If a sample is less than 0.5 grams the sample will not be quantitated and the result should be "sample is unsuitable for quantitative analysis, insufficient amount". These samples will be qualitatively analyzed.

6.2.2.2 If the concentration of a sample is below 10% then the result should be "sample is unsuitable for quantitative analysis, concentration is below the level of quantitation".

6.2.2.3 All calculated results will be reported to the same degree of significance.
6.2.2.4 All results will be rounded using standard rounding rules, i.e. 1-4 down, 6-9 up and 5 to the nearest even number.

7.0.0 Notes and QA/QC

7.1.0 The curve must be linear as defined by a correlation coefficient of 0.998 or better. The correlation coefficient is generated by the Agilent (Hewlett-Packard) Chemstation software.

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

7.3.0 A new curve will be generated before each quantitation sequence. A sequence is defined as a batch(s) run consecutively without the introduction of non-quantitation samples. A batch is defined as up to twenty injections. At the end of each batch a positive control will be run, the results of which must be (+/-) 7% of the stated value. The Relative Percent Difference (RPD) will be calculated for each batch of positive controls:

$$RPD = \frac{|R1-R2|}{E} * 100$$

Where R1 = calculated result of the first positive control run after the generation of the curve.
R2 = calculated result of positive control run at the end of the batch, or sequence if two or more batches are run together.
E = Expected value

The RPD will be less than 14%.

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

7.5.0 A positive control will be analyzed each time a curve is generated. The positive control will come from a source other than what was used to generate the curve. Another in-house standard from a different lot, if available, and prepared by a different analyst is to

be used as the positive control. To a 100ml volumetric flask add approximately 0.1g of methamphetamine, that has been accurately weighed, then dissolve and bring to the mark with internal standard. The positive control is made with the same batch of internal standard as the rest of the run. Aliquot one milliliter into an auto-sampler vial and add sodium carbonate solution.

7.6.0 The accuracy of the curve is validated when the value of the positive control is within (+/-) 7% of the stated value.

7.7.0 The calibration curve, chromatograms and quantitation reports of the positive controls, excel spreadsheets containing RPD (positive controls) calculation results and syringe verification results for each run, and sequence logs will be centrally located with the discipline leader. Chromatogram(s) and quantitation report(s) of all samples, and chromatograms of all applicable blanks are to be kept in the case notes. Chromatograms of standards used to generate the curve do not need to be kept.

7.8.0 For the 1.0 ml syringe weigh 10 replicate aliquots of water at 200ul and 800ul. For the purpose of the calculations, the density of water is 0.998 g/ml. The acceptance criteria are (+/-) 3% of 0.1996 (for 200ul) and 0.7984 (for 800ul) for all measurements.

8.0.0 Uncertainty of Measurement

The (+/-) 7% value was derived from a validation study that took into account this method, different instruments and analysts, as such as long as the constraints described in this method are followed the stated UM value is valid. Before a new analyst or instrument is used on casework it must be demonstrated that they/ it can meet the original target goals of the validation study. The UM value will be reviewed annually and adjusted if necessary.

9.0.0 History

Revision #	Issue or review date	History	Author or Reviewer
0	5/24/02	Original Issue	D.C. Sincerbeaux
1	8/27/02	Add #	D.C. Sincerbeaux
2	1/10/03	Added 7.7 and 7.8	D.C. Sincerbeaux
3	9/30/05	Added 4.4.0, Changed 5.1.0, 5.2.0, 5.3.0, 4.3.0, 7.3.0 and renumbered 7.0. Added 7.10.0	D.C Sincerbeaux
4	8/08/08	added 3.5, 3.6, 5.3, 5.5.0, 5.5.2 6.2, 7.8, 7.9. Changed 4.1, 4.2, 4.4, 6.1, 6.2, 7.1, 7.3, and 7.5	D.C. Sincerbeaux
5	11/20/08	Changed 5.4.0 and 6.0.0	D.C. Sincerbeaux

6	8/11/10	Changed background, scope, 3.5.0, 5.0.0, 5.4.0, 6.2.1, and 7.7.0. Added 6.2.2 and completely replaced 7.8.0 D.C. Sincerbeaux
7	8/15/11	Changed 3.5.0, 5.5.0, 6.2.1 added 6.2.2.3 and 6.2.2.4 D.C. Sincerbeaux
8	12/16/13	Changed 5.4.0, 6.1.0, 6.2.1, 6.2.2.3, 7.8.0, new 8.0.0 D.C. Sincerbeaux
9	12/30/15	Changed the scope adding 2.3.0. Changed 5.5.1 D.C. Sincerbeaux