Technical Procedure for Concentration Determination of Methamphetamine in Liquids via HPLC

1.0 Purpose This procedure specifies the required elements for the preparation and use of the Agilent 1100/1200 series High Performance Liquid Chromatograph (HPLC) to determine the concentration of methamphetamine in liquids.

2.0 Scope This procedure applies to the HPLC systems used in Drug Chemistry at the Raleigh and Western Regional locations of the State Crime Laboratory.

3.0 Definitions

- **Performance verification –** The initial confirmation of the reliability of a previously or externally validated method or instrument.
- **Quality control (QC) check –** Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
- **Reference Material** – Material sufficiently homogeneous and stable, with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Agilent 1100 Series Thermostatted Column Compartment
- Agilent 1100 Series Autosampler
- Agilent 1200 Series Vacuum Degasser
- Agilent 1200 Series Quaternary Pump
- Agilent 1100 Series Diode Array Detector
- Printer or other output device
- PC Data system with HPLC 2D Chemstation software, Version B.02 (or higher upgrade)

4.2 Materials

- Phenomenex Synergi Hydro-RP 4µ C18 Column, 150x4.6mm Solvent Reservoirs (or other interchangeable column)
- 2ml Autosampler vials with 11mm caps
- Filtration System with vacuum access
- Class A Volumetric Flasks
- Funnels
- Class A Graduated Cylinders
- Class A Volumetric Pipettes
- 0.45 µ filters
- 0.45 µ syringe filters
- Syringes

4.3 Commercial Reagents

- Phosphoric acid
- Triethanolamine
- HPLC grade water

- HPLC grade acetonitrile

4.4 Primary Reference Materials - The following reference materials shall be selected from certified reference materials maintained by the laboratory.

- d-Methamphetamine HCl
- d-Amphetamine
- (+/-)3,4-Methylenedioxyamphetamine (MDA)
- (+/-)3,4-Methylenedioxymethampetamine (MDMA)
- (+)-Pseudoephedrine
- (-)-Ephedrine

5.0 Procedure

5.1 Reagents, Standards, and Controls - The reagents, standards, and controls may be prepared in any amount provided that the component ratios are kept constant.

5.1.1 Buffer Preparation

5.1.1.1 Add 22.5 mL of phosphoric acid and 22 mL of triethanolamine to 4 L of HPLC Grade Water.

5.1.1.1.1 The pH of this solution shall be between 2.2 and 2.3. The amount of phosphoric acid may be adjusted to achieve the desired pH.

5.1.1.2 Expiration date is one month from preparation if kept at room temperature.

5.1.1.3 If stored in the refrigerator, the buffer shall have the pH checked before use to ensure that it is still within the specification stated in **5.1.1.1.1**. The expiration date on the buffer stored in the refrigerator will be one year after preparation. Buffer solution shall be used as the mobile phase in the instrument operation.

5.1.2 0.01 N Hydrochloric Acid (HCl)

5.1.2.1 Add 210 µL concentrated hydrochloric acid to approximately 200 mL of deionized water in a 250 mL volumetric flask. The volumes may be changed as long as the ratios remain constant.

5.1.2.2 Mix and dilute to volume with deionized water.

5.1.2.3 Expiration date: One year.

5.1.2.4 Refrigerate

5.1.2.5 QC Check: Test acidic to pH or litmus paper.

5.2 Standard Preparation

5.2.1 Prepare the following concentrations of standard solutions of methamphetamine. The calibration primary standard and the verification primary standard shall be sampled from the different suppliers or different lot numbers with a supplier available at the time of preparation.

5.2.2 Dilute each standard in a 0.01 N HCl solution.

5.2.3 The following weight to volume dilutions are recommendations which may be increased or decreased depending upon the volume of standard needed. However, the ratio of weight to volume shall remain constant.

5.2.4 Make a 20.0 mg/mL stock solution by weighing out 2.0 g into a 100 mL volumetric flask using an analytical balance. Dilute to 100 mL in a flask with a 0.01 N HCl solution.

5.2.5 0.40 mg/mL: Pipet 2.0 mL of the 20.0 mg/mL stock solution into a 100 mL flask. Dilute to 100 mL with a 0.01 N HCl solution.

5.2.6 0.20 mg/mL: Pipet 50.0 mL of the 0.40 mg/mL solution into a 100 mL flask. Dilute to100 mL with a 0.01 N HCl solution.

5.2.7 0.10 mg/mL: Pipet 50.0 mL of the 0.20 mg/mL solution into a 100 mL flask. Dilute to 100 mL with a 0.01 N HCl solution.

5.2.8 0.05 mg/mL: Pipet 50.0 mL of the 0.10 mg/mL solution into a 100 mL flask. Dilute to 100 mL with a 0.01 N HCl solution.

5.2.9 0.01 mg/mL: Pipet 20.0 mL of the 0.05 mg/mL solution into a 100 mL flask. Dilute to 100 mL with a 0.01 N HCl solution.

5.2.10 A 0.40 mg/mL verification standard per 5.2.1 shall be prepared and used to verify the calibration curve. Repeat 5.2.4 and 5.2.5 label solution as a verifier.

5.2.11 A 0.02 mg/mL verification standard per 5.2.1 shall be prepared and used to verify the calibration curve. Using the 0.40 mg/mL solution prepared in 5.2.10: pipet 5 mL of 0.40 mg/mL solution into a 100 mL flask. Dilute to 100 mL with a 0.01 N HCl solution.

5.2.12 The expiration date of the prepared standards shall be one year from the date prepared. All standards will be stored in the refrigerator. However, if a standard fails to produce a valid calibration curve, it shall be discarded and new standards shall be prepared. New standards shall provide accurate and reproducible data before they can be used in analysis (see **5.7**).

5.3 System Check Solution Preparation

5.3.1 Weigh 5 mg (amount may be adjusted dependent on standard availability) of each of these commercial primary standards into a 50 mL flask and dilute with a 0.01 N HCl solution: pseudoephedrine, ephedrine, methamphetamine, amphetamine, MDA, and MDMA.

5.3.2 The expiration date of the system check solution shall be one year from the date prepared. The solution will be stored in the refrigerator. However, if a solution fails to produce valid results, it shall be discarded and a new solution shall be prepared.

5.3.3 The system check solution shall be verified before every run on the instrument.

5.4 Wash Solution

5.4.1 Mix 10 mL of acetonitrile and 10 mL of HPLC grade water to form a 50:50 acetonitrile: HPLC grade water solution.

5.5 Performance Verification for New Instrumentation

5.5.1 New high performance liquid chromatographs shall be installed by a manufacturer representative and shown to meet any manufacturer's requirements.

5.5.2 Performance verification shall be performed by the HPLC Key Operator on new high performance liquid chromatographs prior to being used for casework.

5.5.3 The performance verification shall include a successful calibration, system check solution run, verification standard runs, and any additional runs needed to show the repeatability, linearity, and selection of methamphetamine.

5.6 Instrument Maintenance and Shutdown

5.6.1 Record all maintenance in the maintenance log at the time it is performed.

5.6.2 When the HPLC is taken out of service (e.g., maintenance, malfunction, leaving the direct control of the Laboratory), correct operation shall be demonstrated by running the system check solution.

5.6.3 Suggested routine maintenance

5.6.3.1 This is a recommended maintenance schedule. Instrument use may alter the need for maintenance and shall be performed at the discretion of the HPLC Key Operator or designee.

5.6.3.2 Before and after each full run, the column shall be flushed with 100 % acetonitrile until a stability of the baseline is reached.

5.6.3.3 If the instrument is not in use for approximately three months, the buffer line shall be placed into a methanol reservoir.

5.6.4 Shutdown

5.6.4.1 If the instrument shuts down, this shut down shall be noted in the instrument log on the day this is noticed. After successfully bringing the instrument back on line with a stable baseline, an acceptable system check solution shall be completed before any calibration standards, verification standards, or case samples are analyzed.

5.7 Calibrations

5.7.1 The Agilent High Performance Liquid Chromatograph shall be calibrated with every run. Performance verification shall be performed prior to the analysis of any casework.

5.7.2 The first injection is the six component system check solution prepared in **5.3**.

5.7.3 All compounds shall be effectively resolved from the methamphetamine peak, with the closest peak to methamphetamine at a resolution greater than 1.5.

5.7.4 Once the previous injection meets all specifications, the analysis of calibration standards, verification standards, and samples may begin.

5.7.5 The calibration shall consist of double injections of the 0.01, 0.05, 0.10, 0.20 and 0.40 mg/mL solutions.

5.7.6 The correlation coefficient produced by these standards shall be greater than 0.995.

5.7.7 The final injections are the 0.02 mg/mL and 0.40 mg/mL verification standards. These standards shall be entered into the sample table as a sample, not a standard. The result shall be within +/- 5 % of the true concentration of the standard. This verifier shall be considered a positive control.

5.7.8 Records

5.7.8.1 The calibration data shall include the lot numbers of the calibrations standards and the system check solution standards. The chromatograms for each calibration standard shall have the coefficient of determination (r^2) values.

5.7.8.2 Record each calibration in the instrument log with the date, lot number of primary standards used (including those from the system check solution), the expiration date of the calibration run, and the operator initials.

5.7.8.3 Record any calibrations that do not meet the requirements in the instrument log.

5.8 Sampling

5.8.1 Analysis samples shall be obtained as a representation from the entire sample. Sampling shall be performed as follows:

5.8.1.1 For all liquids, a reasonable amount of sample shall be seized from the site by the processing Forensic Scientist, depending on the amount of liquid available at the scene, and the amount needed to perform qualitative analysis.

5.9 Instrument Parameters

- Oven temperature: 55 °C
- Flow rate: 0.8 mL/min
- Injection amount: 5.0 µL

- Run time: approximately 17.5 minutes
- Detector: diode array detector 210 nm Bw10, reference 550 nm Bw 100
- Mobile phase: 93 % buffer, 7 % acetonitrile
- Sample solvent: 0.01 N HCl
- Wash solution: 50 % HPLC grade acetonitrile, 50 % HPLC grade water

5.10 Application of Procedure on Evidence

5.10.1 Sample Preparation

5.10.1.1 For single-layered liquids, pipette a 1.0 mL aliquot of the liquid into a volumetric flask using a volumetric pipette. 100 mL flask size is suggested, but may be modified based on sample size and the pH of the sample solution.

5.10.1.2 Each layer of a two-layered liquid shall be analyzed separately. After separating the two-layered liquid, refer to **5.10.1** for sample preparation.

5.10.1.3 Two dilutions of each case sample or each liquid if two layers are present shall be prepared using a 0.01 N HCl solution as the dilution solvent.

5.10.1.4 The dilutions shall be mixed thoroughly before sampling to put on the instrument. For organic samples, a bi-layer will form once the 0.01 N HCl is added. The aqueous layer shall then be sampled out for analysis.

5.10.1.5 Ensure that an adequate amount of the dilution solvent has been added to produce an acidic solution.

5.10.1.6 Filter approximately 2 mL of each solution through a 0.45 µ syringe filter before injection.

5.10.2 Sample Injection

5.10.2.1 Place a 50:50 acetonitrile:water solution in the designated autosampler well for the instrument to perform self-cleaning on the needle and injection port after each injection.

5.10.2.2 Inject 5.0 µL of the blank solution (0.01 N HCl) before each sample injection, into the injector port. Observe the chromatogram for any interferences. This shall be a negative control.

5.10.2.3 Inject 5.0 µL of the filtered sample.

- Prepare each sample in duplicate.
- Analyze each preparation in duplicate.

5.10.2.4 When data collection is complete, observe the chromatograms for the following:

- The results shall be the concentration of methamphetamine hydrochloride in mg/mL.

- The calculated concentrations shall differ no more than 3 % from one another.
- If any preparation falls outside of these limits, the dilution shall be prepared again and a new analysis shall be performed.

5.10.2.5 Reporting

5.10.2.5.1 Include the calibration results with the reported r^2 values, the system check solution results showing the resolution of the peaks is greater than 1.5, the verification results, and the sample results into the FA case record.

5.11 Calculations - An average of the results of the four injections shall be calculated and that concentration will be the reported value.

5.11.1 For one-layered liquids, the concentration determined, as outlined in **5.10.2.4**, shall be the final concentration reported.

5.11.2 For two-layered liquids, as stated in **5.10.1.2**, each layer shall be worked separately. The concentration for each layer determined, as outlined in **5.10.2.4**, shall be the final concentration reported.

5.12 Uncertainty of Measurement - See the Drug Chemistry Section Procedure for Uncertainty of Measurement.

6.0 Limitations - N/A

7.0 Safety

7.1 Use caution such as gloves and a fume hood when handling acetonitrile, triethanolamine, and phosphoric acid to avoid eye and skin contact. Use caution when handling the 0.01 N HCl solution as it has acidic properties.

8.0 References

"Chromatographic Quantitation of Methamphetamine." North Carolina State Bureau of Investigation Raleigh and Western Regional Laboratories, 2007.

Weston, Robert. "HPLC validation for use in quantification of methamphetamine in liquids." Oklahoma State Bureau of Investigation-Controlled Substances Unit, 2008.

9.0 Records

- FA System Case files
- HPLC Logbook
- HPLC Activity Log
- HPLC Maintenance Log

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document