Technical Procedure for High Performance Liquid Chromatography

1.0 Purpose - This procedure specifies the required elements for the calibration and use of the Agilent 1100/1200 series High Performance Liquid Chromatograph (HPLC).

2.0 Scope – This procedure applies to the HPLC systems used in Drug Chemistry at the Raleigh and Western locations of the State Crime Laboratory. This procedure shall be used for the quantitation of methamphetamine in solid drug cases. Quantitation shall be performed only for Federal prosecutions.

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)
- pH meter

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 (HPLC)
- 0.45 µ syringe filters (HPLC)
- 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.

5.2 Standard Preparation

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

5.2.2 Dilute each standard in a 90:10 solution of buffer:acetonitrile.

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 1.0 mg/mL: Weigh 50 mg of methamphetamine into a 50 mL flask using an analytical balance. Dilute to 50 ml with a 90:10 buffer:acetonitrile solution.

5.2.5 0.75 mg/mL: Weigh approximately 37.5 mg of methamphetamine into a 50 mL flask using an analytical balance. Dilute to 50 mL with a 90:10 buffer:acetonitrile solution.

5.2.6 0.50 mg/mL: Pipet 25.0 mL of the 1.0 mg/ml standard into a 50 mL flask. Dilute to 50 mL with a 90:10 buffer:acetonitrile solution.

5.2.7 0.25 mg/mL: Pipet 25.0 mL of the 0.50 mg/mL standard into a 50 mL flask. Dilute to 50 mL with a 90:10 buffer:acetonitrile solution.

5.2.8 0.05 mg/mL: Pipet 10.00 mL of the 0.25 mg/mL standard into a 50 mL flask. Dilute to 50 mL with a 90:10 buffer:acetonitrile solution.

5.2.9 A 1.0 mg/mL verification standard shall be prepared per 5.2.1 and used to verify the calibration curve. Repeat **5.2.4** and label the solution as a verifier.

5.2.10 A 0.50 mg/mL standard shall be prepared per **5.2.1** and used to verify the calibration curve. Repeat 5.2.6 and label the solution as a verifier. (The 1.0 mg/mL verifier solution is used in this preparation.)

5.2.11 There expiration date of 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 may be used in analysis (refer to **5.7**).

5.3 System Check Solution Preparation

5.3.1 Weigh 5 mg (amount may be adjusted dependant on standard availability) of each of these commercial standards primary standards into a 50 mL flask and dilute with a 90:10 buffer:acetonitrile solution: (+)-pseudoephedrine, (-)-ephedrine, d-methamphetamine, d-amphetamine, (+/-)MDA, and (+/-)MDMA.

5.3.2 The expiration date of the system check solution shall be one year from the date prepared. The solutions 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 by 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, then 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 Calibration

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 which consists of (+)-pseudoephedrine, (-)-ephedrine, d-methamphetamine, d-amphetamine, (+/-)MDA, and (+/-)MDMA.

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, analysis of calibration standards, verification standards and samples may begin.

5.7.5 The calibration shall consist of double injections of the 0.05, 0.0.25, 0.50, 0.75 and 1.0 mg/mL solutions shall be performed after the system check.

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

5.7.7 The final injections are the 0.50 and the 1 mg/mL verification standard. These verification standards shall be entered into the sample table as samples, not as standards. 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 of each component.

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 Samples analyzed from unknowns shall represent the entire exhibit. Sampling shall be performed as follows:

5.8.2 Single items

5.8.2.1 If the sample is 1 ounce or less, homogenize the entire amount, take the two samples required and return the unused portion to the evidence container.

5.8.2.2 If the sample is more than 1 ounce, take approximately 10 % to homogenize. Take the two samples required and return the unused portion to the evidence container.

5.8.2.3 For large samples, more than 500 grams, either a core sample or combined samples taken from multiple locations that equate to 10 % of the total weight should be used for the homogenizing process.

5.8.3 Multiple packages in a single item

5.8.3.1 Single items that contain multiple packages shall be analyzed qualitatively to determine if combining is appropriate. If qualitative analysis shows differences, the packages will be treated as sub-items and independently analyzed for quantitation. If no differences are observed, the packages may be combined to form a composite prior to homogenization.

5.9 Instrument Parameters for use

- 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 210nm Bw10, reference 550nm Bw 100

- Mobile phase: 93 % buffer, 7 % acetonitrile
- Sample solvent: 90 % buffer, 10 % acetonitrile
- 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 The sample shall be prepared by homogeneously grinding and mixing in a mortar using a pestle.

5.10.1.2 Once the sample is homogenous, filter through a 20 mesh sieve. The filtered powder shall be used to make the sample dilution.

5.10.1.3 Weigh on an analytical balance approximately 150 mg of sample.

5.10.1.4 Prepare the dilution in a 200 mL volumetric flask or a volume appropriate to the concentration, using the 90:10 buffer:acetonitrile solution as the solvent.

5.10.1.5 Thoroughly mix the solution.

5.10.1.6 Two dilutions of each case sample shall be prepared. It is recommended that at least 100 mg of sample be used for each dilution; therefore, it may be necessary to perform serial dilutions.

5.10.1.7 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 a 5.0 µL blank of the (90:10 buffer:acetonitrile) solution before each sample set. Observe the chromatogram for any interference. This shall be a negative control.

5.10.2.3 Inject 5.0 µL of the filtered sample.

5.10.2.3.1 Prepare each sample in duplicate.

5.10.2.3.2 Analyze each preparation in duplicate.

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

5.10.2.4.1 The percentages shall differ no more than 3 % from one another.

5.10.2.4.2 If any preparation falls outside of these limits, the dilution shall be prepared again and a new analysis will 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 percentage shall be the reported value.

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

6.0 Limitations - N/A

7.0 Safety - Use caution such as gloves and a fume hood when handling acetonitrile, triethanolamine, and phosphoric acid to avoid eye and skin contact.

8.0 References

Moffat, Osselton, and Widdop. *Clarke's Analysis of Drugs and Poison.* 3rd Edition. Volume 2, 2004.

Agilent Chemstation: Understanding Your Chemstation, 02/06 Ed. Agilent, 2006.

Agilent 1200 Series Quaternary Pump: Reference Manual, 02/06 Ed. Agilent, 2006.

Agilent 1100 Series Standard, Micro, and Preparative Autosamplers: Reference Manual, 05*/04 Ed.* Agilent, 2004.

Agilent 1100 Series Diode Array and Multiple Wavelength Detector SL: User Manual, 06/05 Ed. Agilent, 2005.

Agilent 1200 Series Vacuum Degasser: Reference Manual. 02/06 Ed. Agilent, 2006.

Agilent 1100 Series Thermostatted Column Compartment: Reference Manual, 05/04 Edition: Agilent, 2004.

9.0 Records

- See specific sections above where applicable.
- HPLC Activity Log
- HPLC Maintenance Log

10.0 Attachments – N/A

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