Name of Instrumental Procedure:

Use of the Agilent 1100/1200 series HPLC to Determine the Concentration of Methamphetamine in Liquids

Suggested Uses:

Concentration determination of Methamphetamine in Liquids

Apparatus:

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
Hewlett Packard LaserJet printer
PC Data system with HPLC 2D Chemstation software, Version B.02 (or higher upgrade)
Phenomenex Synergi Hydro-RP 4μ C18 Column, 150x4.6mm
Agilent HPLC Reference Manuals
Solvent Reservoirs
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

Reagents and Standards:

Phosphoric Acid
Triethanolamine
HPLC Grade Water
HPLC Grade Acetonitrile
d,Methamphetamine HCl
d,Amphetamine
(+/-)3,4-Methylenedioxyamphetamine (MDA)
(+/-)3,4-Methylenedioxymethampetamine (MDMA)
(+)-Pseudoephedrine
(-)-Ephedrine

Procedure for use:

Oven Temperature: 55°C
Flow Rate: 0.8 ml/min
Injection Amount: 5.0 µl
Run Time: 20 minutes
Detector: Diode Array Detector 210nm Bw10, Reference 550nm Bw 100
Mobile Phase: 93% Buffer, 7% Acetonitrile
Sample Solvent: 0.01N HCl
Wash Solution: 50% HPLC Grade Acetonitrile, 50% HPLC Grade Water

Buffer Preparation:

Into 4L of HPLC Grade Water, add 22.5 ml of Phosphoric Acid and 22 ml of Triethanolamine. The pH of this solution should be 2.2-2.3. The amount of phosphoric acid can be adjusted to achieve the desired pH. After mixing the solution, filter and degas through a 0.45 µ filter. Expiration is one month from preparation if kept at room temperature and no expiration if kept in the refrigerator.

Standard Preparation:

Prepare the following concentrations of standard solutions of Methamphetamine from a commercial standard that contains a certificate of analysis. Dilute each standard in a 0.01 N HCl solution. The following weight to volume dilutions are suggestions. They can be increased or decreased depending upon the volume of standard needed. However, the ratio of weight to volume should remain constant.

Make a 20.0 mg/mL stock solution by weighing out 2.0 g into a 100mL volumetric flask using an analytical balance, calibrated prior to weighing. Dilute to 100.0ml in a Class A flask with a 0.01N HCl solution.

0.40 mg/ml: Pipet 2.0mL of the 20.0 mg/mL stock solution into a 100.0mL flask. Dilute to 100.0 ml in a Class A flask with a 0.01N HCl solution.
0.20 mg/ml: Pipet 50.0mL of the 0.40 mg/mL solution into a 100.0mL flask. Dilute to 100.0 ml in a Class A flask with a 0.01N HCl solution.
0.10 mg/ml: Pipet 50.0mL of the 0.20 mg/mL solution into a 100.0mL flask. Dilute to 100.0 ml in a Class A flask with a 0.01N HCl solution.
0.05 mg/ml: Pipet 50.0mL of the 0.10 mg/mL solution into a 100.0mL flask. Dilute to 100.0 ml in a Class A flask with a 0.01N HCl solution.
0.01 mg/ml: Pipet 20.0mL of the 0.05 mg/mL solution into a 100.0mL flask. Dilute to

100.0 ml in a Class A flask with a 0.01N HCl solution.
These standards have no expiration as long as they are stored in the refrigerator. However, if the standards are ran on the instrument and seem to be degraded at all, they will all need to be remade and samples will not be ran until the standards are giving reproducible and accurate data.

System Check Solution Preparation:

Accurately weigh 25 mg of each of these commercial standards into a 50 ml Class A flask and dilute with a 0.01N HCl solution: Pseudoephedrine, Ephedrine, Methamphetamine, Amphetamine, MDA, and MDMA. If this amount of material is not available, then the weight can be lowered as long as the data shows the appropriate selectivity. This standard has no expiration as long as they are stored in the refrigerator. However, if the standards are ran on the instrument and seem to be degraded at all, they will all need to be remade and samples will not be ran until the standards are giving reproducible and accurate data.

Calibration of the Instrument:

The calibration procedure for the Agilent High Performance Liquid Chromatograph is performed for each analysis run. The first injections are the 0.01 and 0.40 mg/ml standard which are injected as samples. The result should be within +/- 5% of the true concentration of the standard. (Calculation is [Observed value - True value]/True value *100). If the concentration is not within the specified limits, the instrument should be checked for problems and no further analysis should be performed until the criteria of this standard is met. If no problems are found with these standards, then inject the six component system check solution. This solution is a mixture of Pseudoephedrine, Ephedrine, Methamphetamine, Amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyamphetamine (MDA). The injection should show that all of the compounds are effectively resolved from the Methamphetamine peak. The resolution of the Methamphetamine peak compared to the closest peak should be greater than 1.5. Once the previous injections pass all requirements, the calibration can be started by making duplicate injections of each of the following standards: 0.01, 0.04, 0.10, 0.20, and 0.40 mg/ml. The correlation coefficient produced by these standards should be greater than 0.999.

Sample Preparation:

Analysis samples should be obtained as a representation from the entire sample. The guidelines for sampling are as follows: for all liquids, an appropriate amount of sample should be seized from the scene by the processing analyst. For one-layered liquids, the sample is prepared by pipetting a 1.0mL aliquot of the liquid into a Class A volumetric flask. For two-layered liquids, the top and bottom layers are treated as separate

samples and the procedure for one-layered liquids is then followed. Two dilutions of each case sample should be prepared using a 0.01N HCl solution as the dilution solvent. The dilutions should be mixed thoroughly before sampling to put on the instrument, as two layers may form in the flask depending on the solvent system of the sample. Typically a 100mL flask would be used, but depending on availability and amount of material in sample, other size flasks would be fine. Once the dilutions are made, each preparation needs to be filtered through a 0.45 μ syringe filter before injection.

Sample Injection

A. A wash solution should be placed in a designated autosampler well for the instrument to perform self-cleaning on the needle and injection port after each injection. This solution is a 50:50 Acetonitrile:Water solution.

B. Inject 5.0 μl of the blank solution (0.01N HCl) before each sample injection, into the injector port. Observe the chromatogram for any interferences.

C. 5.0 μl of the filtered sample can then be injected. Each sample is prepared in duplicate and each preparation is analyzed in duplicate.

D. When data collection is complete, observe the chromatograms for methamphetamine. Each of the injection results will yield a concentration in mg/mL of methamphetamine. The concentrations between the like dilutions should differ no more than +/- 3% from one another. If any preparation falls outside of these limits, the dilution will be prepared again and a new analysis will be performed. An average of the results of the injections will be calculated and that concentration will be the reportable value.

Safety Concerns:

Use caution when handling Acetonitrile, Triethanolamine, and Phosphoric Acid to avoid eye and skin contact. Also, use caution when handling the 0.01N HCl solution as it has acidic properties.

Literature References:

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

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