Biochemical Diagnostics, Inc.
180 Heartland Blvd, Edgewood, NY 11717● Phone (800) 223-4835
Fax (631) 595-9204 ● www.biochemicaldiagnostics.com

DETECTABUSE® "NO VACUUM" GRAVITY SERIES GV-65 / GV-65C METHOD FOR THE ANALYSIS OF AMPHETAMINE, METHAMPHETAMINE, 3,4-METHYLENEDIOXYAMPHETAMINE (MDA), 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA), 3,4-METHYLENEDIOXYETHYLAMPHETAMINE (MDEA) IN SERUM, ORAL FLUID OR URINE BY GC/MS

July 05, 2011

Please see Notes and Supplemental Information before proceeding

SAMPLE PREPARATION

1. Add 1.0 mL of sample to a 16 x 100 mm disposable culture tube.
2. Add 100 uL (5000 ng/mL) of Amphetamine D-11, Methamphetamine D-14, MDA - D5, MDMA - D5 and MDEA -D6.
3. Add 1.0 mL of 0.25M Phosphate Buffer pH 6.
4. Add 0.5 mL of 10% Sodium Metaperiodate in H2O Mix well.
5. Incubate at room temperature for 20 min.
6. Adjust pH to 2 with ≈ 0.1 – 0.2 mL of 10% HCl.
7. Proceed to sample extraction.

COLUMN CONDITIONING – *ALL LIQUIDS FLOW BY GRAVITY*

(Follow Column Conditioning procedure for <u>EITHER</u> GV-65 or GV-65C columns.

Column Conditioning and Activation of Cation Function using <u>GV-65</u> Columns.

1. Wash column with 1.0 mL of Methanol.
2. Add 1.0 mL of a Sodium Bisulfite solution to each column.
   Prepare by dissolving 5 grams of Sodium Bisulfite in 100 mL of a (1:1) mixture of H2O:0.25M Phosphate Buffer, pH 6.0. Prepare monthly. (Store refrigerated)
3. Wash column with 1 mL deionized water.
4. Proceed to Sample Extraction within 20 min. of column conditioning.

Column Conditioning using <u>GV-65C</u> Columns

Note: *The GV-65C column is manufactured with the cation exchanger and does not require the addition of sodium bisulfite.*

1. Wash column with 1.0 mL of Methanol.
2. Wash column with 1 mL deionized water.
3. Proceed to Sample Extraction within 20 min. of column conditioning.

SAMPLE EXTRACTION

1. Pour samples onto preconditioned column.
2. Wash column with 2.0 mL 0.1M glacial Acetic Acid.
3. Wash column with 3.0 mL of deionized water.
4. Wash column with 1.0 mL of Methanol.
5. Wash column with 1.0 mL Ethyl Acetate.

Proceed to Sample Elution.

SAMPLE ELUTION

1. Sample elution is done outside of the vacuum box.
2. Place the column mounting plate on the elution rack loaded with an appropriate number of 12 x 75 mm or 16 x 100 mm culture tubes. Make sure that the hole pattern on the plate matches the hole pattern on the rack.
3. Add 1.5 mL of n-Butylchloride:Ethyl Acetate (80:20) +4% Triethylamine (TEA) to each column.

EVAPORATION

1. Add 200 uL of 0.05% HCL in Methanol to each tube.
2. Evaporate just to dryness at 40^0 C.

DERIVATIZATION - The following are examples

Using HFBA
1. Add 50 µL Ethyl Acetate and 50 µL Heptafluorobutyric Anhydride (HFBA) to each dried column eluate, cap tubes and gently mix the contents.
Amphetamine/Methamphetamine: Heat at 70^0C for 20 mins.
Amphetamine/Methamphetamine +MDA, MDMA, MDEA: Heat at 75^0C for 60
Cool..
2. Evaporate just to dryness at 40°C and reconstitute with 100 µL Ethyl Acetate.
3. Vial and cap.

Using the 4-CB Derivative:
1. Add 100 µL of 4-Carbethoxyhexafluorobutyl Chloride which has been diluted 1:100 with n-Butylchloride to each dried column eluate and cap tubes. Gently mix the contents and incubate at 75°C for 30 min. The derivatization of MDEA MDA, MDMA, MDEA, is improved at 90°C for 30 minutes.
2. Add 300 µL Absolute Ethanol and incubate at 50°C for 15 min.
3. Evaporate just to dryness at 40°C. Reconstitute with 100 µL Ethyl Acetate.
4. Vial and cap.

MSD SIM PROGRAM

HFBA

Drug	Ions Monitored
Amphetamine-D11	128, 244
Amphetamine	91, 118, 169, 240
Methamphetamine-D14	213, 261
Methamphetamine	118, 210, 254
MDA-D5	167, 244, 380
MDA	162, 240, 375
MDMA-D5	213, 258
MDMA	162, 210, 254
MDEA-D6	165, 244. 274
MDEA	162, 240, 268,

4-CB

Drug	Ions Monitored
Amphetamine-D11	270, 298
Amphetamine	248, 266, 294
Methamphetamine-D14	287, 315
Methamphetamine –D 5	284, 312
Methamphetamine	262, 280, 308
MDA-D5	167, 270
MDA	162, 248, 266
MDMA-D5	167, 284, 312
MDMA	162, 280, 308
MDEA-D6	328, 165
MDEA	322, 294, 276

NOTES:

1. **SAMPLES AND WASHES** – Allow all samples and washes to gravity flow completely through the resin bed before adding the next liquid.

2. **INTERNAL STANDARDS** may be made up in an aqueous matrix. When adding an internal standard dissolved in an organic solvent to a sample, the solvent must not exceed 2% of the final prepared sample.

3. **ANALYSIS OF ALL SYMPATHOMIMETIC AMINES** the oxidation step must be omitted

4. **TURBID SAMPLES** may need to be centrifuged.

5. **RINSE SOLVENTS** should be delivered to the top part of the column to better remove the aqueous. Prepare fresh daily.

6. **ELUTION SOLVENTS** with the TEA should be made fresh daily.

7. **POLAR SOLVENTS** used (e.g. acetonitrile and ethyl acetate) may absorb moisture. Flush bottles with nitrogen, keep stock bottles full or use sodium sulfate to minimize moisture.

8. **ADDITION** OF 0.05% HCl in Methanol is critical to prevent volatile loss of drug.

9. **IDEAL FRAGMENTS** should be determined by full scans of neat, derivatized standards.

10. **RECOMMENDED CAPILLARY COLUMNS** for adequate partitioning of all amphetamines would be 5% or 35% phenyl columns.

This method is a preliminary procedure for investigational use only. Although it has performed well in our laboratory the method must be validated by your laboratory before it is used to report patient values. We would appreciate your comments on its performance and welcome your suggestions for improvements or enhancements.