



**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**

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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  $\mu$ L (5000 ng/mL) of Amphetamine-D 8 or D11, Methamphetamine- D-11 or D-14, MDA - D5, MDMA - D5 and MDEA -6.
3. Add 1.0 mL of 0.25M Phosphate Buffer pH (6).
4. Add 0.4 mL of 10% Sodium Metaperiodate in H<sub>2</sub>O Mix well.
5. Incubate at room temperature for 20 min.
6. Adjust pH to 2 with 10% HCl.
7. Proceed to sample extraction.

**COLUMN CONDITIONING – ALL LIQUIDS FLOW BY GRAVITY**

(Follow Column Conditioning procedure for **EITHER** GV-65 or GV-65C columns.

**Column Conditioning and Activation of Cation Function using GV-65 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 H<sub>2</sub>O: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 GV-65C 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. 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 – ALL FLOWS BY GRAVITY**

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) with 4% Triethylamine (TEA)\* to each column.

**EVAPORATION**

1. Add 200  $\mu$ L of 0.05% HCl to each tube.
2. Evaporate just to dryness at 40 C.

**DERIVATIZATION** - The following are examples

**Using HFBA**

1. Add 50  $\mu$ L Ethyl Acetate and 50  $\mu$ L Heptafluorobutyric Anhydride (HFBA) to each dried column eluate, cap tubes and gently mix the contents. Heat at 70°C for 20 mins. Cool
2. Add 100  $\mu$ L of 0.05% HCl in MeOH to each tube.
3. Evaporate just to dryness at 40°C and reconstitute with 100  $\mu$ L Ethyl Acetate.
4. Vial and cap.

**Using the 4-CB Derivative:**

1. Add 100  $\mu$ L of 4-Carboxyhexafluorobutyl 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.
2. Add 300  $\mu$ L Absolute Ethanol and incubate at 50°C for 15 min.
3. Evaporate just to dryness at 40°C. Reconstitute with 100  $\mu$ L Ethyl Acetate.
4. Vial and cap.

## MSD SIM PROGRAM

Drug	<u>HFBA</u> Ions Monitored
Amphetamine-D5	123, 244
Amphetamine D-11	XXX, XXX
Amphetamine D-8	XXX, XXX
Amphetamine	91, 118, 169, 240
Methamphetamine-D-11	213, 258
Methamphetamine	118, 210, 254
MDA-D-5	167, 244
MDA	162, 240, 375
MDMA-D-5	213, 258
MDMA	162, 210, 254
MDEA-D-6	274, 409
MDEA	162, 240, 268,

Drug	<u>4-CB</u> Ions Monitored
Amphetamine-D-11	
Amphetamine D-6	270, 298
Amphetamine	248, 266, 294
Methamphetamine-D-11	284, 312
Methamphetamine	262, 280, 308
MDA-D-5	167, 270
MDA	162, 248, 266
MDMA-D-5	167, 284
MDMA	162, 280, 308
MDEA-D-6	328, 165
MDEA	322, 294, 162

## NOTES:

1. **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 5% of the final prepared sample.
2. **ANALYSIS OF ALLSYMPATHOMIMETIC AMINES** the oxidation step must be omitted
3. **TURBID SAMPLES** may need to be centrifuged.
4. **RINSE SOLVENTS** should be delivered to the top part of the column to better remove the aqueous. Prepare fresh daily.
5. **ELUTION SOLVENTS** with the TEA should be made fresh daily.
6. **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.
7. **ADDITION** OF 0.05% HCl in MeOH is critical to prevent volatile loss of drug.
8. **IDEAL FRAGMENTS** should be determined by full scans of neat, derivatized standards.
9. **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.*