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 PARENT THC AND DELTA-9-THC-CARBOXYLIC ACID IN ORAL FLUID, HYDROLYZED URINE OR SERUM BY GC/MS

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Please see Notes and Supplemental Information before proceeding

ORAL FLUID OR URINE SAMPLE HYDROLYSIS

1. Pipette 2.0 – 3.0 mL of sample into a 16 x 100 mm disposable glass tube.
2. Add appropriate amount each of Delta-9-THC-COOH-D9 as internal standard.
3. Add 10N KOH to each sample (100 µL/mL sample). Vortex mix.
4. Heat in a 60˚C water bath for 20 min.

SAMPLE PREPARATION (Following Hydrolysis)

1. Add Glacial Acetic Acid (200 µL /mL sample) and 1 mL 0.2M Acetate Buffer, pH 4.0 to each hydrolyzed sample. Mix. Verify a pH of 3-4

SERUM SAMPLE PREPARATION (Without Hydrolysis)

1. Add 3.0 mL of 0.2M Acetate Buffer pH 4.0 to each sample, Mix. Verify a pH of 3-4.
2. Add appropriate amount each of deuterated Delta-9-THC-COOH-D_9 and Delta-9-THC-D_3 as internal standard.

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

SAMPLE EXTRACTION

1. Pour samples onto preconditioned column.
2. Add 1.0 mL Acetonitrile:Water (15:85) containing 2% Triethylamine (TEA)* to each column.
3. Add 3 mL of deionized H2O.
4. Add 1.0 mL of 70% Methanol containing 0.5% Acetic Acid to each column.
5. Vacuum at >7 mm Hg for 3 minutes.

SAMPLE ELUTION

1 Place the column mounting plate on the elution rack loaded with corresponding labeled 12 x 75 mm or 16 x 100 mm glass test tubes. Match the pattern of the mounting plate to the elution rack.
2. Add 1.0 mL Ethyl Acetate:Isopropanol (85:15) to each column and allow solvent to flow through the columns by gravity into the test tubes.
3. Dry under N2 or argon between 50-60˚C.

DERIVATIZATION

A. Using BSTFA (with 1% TMCS)

1. Add 50 µL Ethyl Acetate, Then 50 µL BSTFA containing 1% Trimethylchlorosilane (TMCS).
2. Mix and incubate the mixture @ 70˚C for 20 min.
3. Cool, vial and cap.

B. Using MTBSTFA (with 1% TBDMCS)

1. Add 50 µL Ethyl Acetate, and 50 µL MTBSTFA containing 1% TBDMCS.
2. Mix and incubate the mixture @ 70˚C for 20 min.
3. Cool, vial and cap.

MSD SIM PROGRAM

BSTFA w/TMCS	**Ions**
Drug	**Monitored**
Delta 9-THC	303, 371, 386
Delta-9-THC-D9	312, 380, 395
Delta-9-THC-COOH	371, 473, 488
Delta-9-THC-COOH-D9	380, 479, 497

MTBSTFA w/TBDMCS	**Ions**
Drug	**Monitored**
Delta-9-THC-COOH	413, 515, 572
Delta-9-THC-COOH-D9	524, 581

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-** When preparing the Internal Standard, the quantity added per mL of sample should approximate the cutoff value of the compound(s) being tested for. The internal standard can almost always be prepared in an aqueous matrix. If prepared in an organic solvent the solvent must not exceed 3% of the final prepared sample.

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 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. **AIR TRAPPED** within the column bed or frits may prevent the liquids from eluting freely by gravity flow. Tapping the column mounting plate onto the vacuum box should initiate flow.

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

9. **RECOMMENDED CAPILLARY COLUMNS** for adequate partitioning are 100% polydimethylsiloxane, 5% phenyl and 35% phenyl

10. **METHANOL** may be substituted for Acetonitrile in the 15:85 Wash Solution. Stability is the same.

11. **A DRYING TEMPERATURE** below 50^{o}C may result in a slightly acid residue which can adversely affect the derivatization.