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

DETECTABUSE® "NO VACUUM" GRAVITY GV-65 / GV-65C METHOD FOR THE ANALYSIS OF BUPRENORPHINE AND NORBUPRENORPHINE IN URINE, SERUM OR ORAL FLUIDS BY GC/MS

Please see Notes and Supplemental Information before proceeding

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SAMPLE PREPARATION-
Enzymatic Hydrolysis of Urine
1. Pipette 1.0 – 2.0 mL of sample.
2. Add appropriate amount of D-4 Buprenorphine and D-3 Norbuprenorphine internal standard(s) to each sample tube.
3. Add approximately 5000 units of Beta-Glucuronidase,(e.g. Helix Pomatia) per mL of sample. Add 0.5 mL of 0.2M Acetate Buffer pH 5.
4. Mix gently and incubate at 50 - 60°C for 2 hours.
5. Adjust pH to 2 with 10% HCl.
7. Centrifuge for 3 minutes at 3500 RPM.

Measurement of Buprenorphine in Serum or Oral Fluid

1. Pipet serum or oral fluid sample (typically 0.1 - 2.0 mL) into a 13 x 100 mm borosilicate test tube.
2. Add appropriate amount of internal standard into each tube.
3. Add 1.0 mL of 1% HCL per mL of sample and mix.
4. If Cloudy or precipitated centrifuge for 3 minutes at 3000 RPM.

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 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. Decant prepared samples onto columns.
2. Wash columns with 3.0 mL of 0.01% HCl.
3. Wash columns with 2.0 mL of Methanol.
4. Add 1.0 mL Ethyl Acetate.

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 borosilicate glass test 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: Triethylamine (TEA)* (76:20:4) to each column.
4. Dry under N2 or argon at less than 50°C.

Derivatization - Using MSTFA
1. To each dried extract add 50 µL Ethyl acetate, vortex mix, then add 50 µL MSTFA.
2. Mix and incubate the mixture @ 70°C for 45 min.
3. Allow the mixture to come to room temperature
4. Transfer to a vial and cap.

Suggested GC oven parameters:.

15 meter DB-5
Start program @ 150ºC and hold 0.5 min. Ramp at 30ºC/min. to 310ºC and hold for 2 mins. Retention for Buprenorphine is appox. 8 mins.

<u>MSD SIM PROGRAM - MSTFA</u>

Drug	Suggested Ions Monitored
Buprenorphine-D-4	454, 480
Buprenorphine	450, 451, 482
Norbuprenorphine-D-3	503, 504
Norbuprenorphine	500, 501, 502

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

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 5% 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.
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. **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.