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

DETECTABUSE® GRAVITY SERIES GV-65 / GV-65C METHOD FOR THE ANALYSIS OF BARBITURATES IN URINE BY GC/MS

MAY 2010

Please see Notes and Supplemental Information before proceeding

SAMPLE PREPARATION

1. Add 1.0 -2.0 mL of sample to a 16 x 100 mm disposable glass culture tube.
2. Add 100 ng of Phenobarbital-D5 per mL of sample
3. Add 3.0 mL of 0.25M Phosphate Buffer, pH 9.1. Vortex mix.

COLUMN CONDITIONING - *ALL LIQUIDS FLOW BY GRAVITY*

Column Conditioning

1. Wash column with 1.0 mL of Methanol.
1. Wash column with 1.0 mL of deionized H2O
3. Proceed to Sample Extraction within 20 min. of column conditioning.

SAMPLE EXTRACTION

1. Pour samples onto preconditioned column.
2. Wash column with 3.0 mL of deionized water.
3. Wash with 1.0 mL of DI Water:Methanol (60:40).
4. Dry the columns by applying vacuum adjusted to at least 7" Hg for 5 minutes. (Test by momentarily placing the heel of hand over the column top. A strong pull should be felt through the column).

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 tubes. Make sure that the hole pattern on the plate matches the hole pattern on the rack.
2. Add 1.0 mL of n-Butylchloride with 4% Triethylamine (TEA). Make fresh daily.
3. Dry under N2 or argon at less than 50°C.

ON-COLUMN DERIVATIZATION

1. To each dried extract add 50 µL ethyl acetate and 50 µL Methylating reagent; such as 0.2M Trimethylphenyl Ammonium Hydroxide (TMPAH) in Methanol
2. Transfer to vials with inserts and cap.

MSD SIM PROGRAM Drug	**Ions Monitored**
Butalbital	138,181, 195, 196,
Butabarbital	112, 169, 184, 211
Amobarbital	112, 169, 184, 239
Pentobarbital	112, 169, 184, 225
Secobarbital	181, 195, 196, 266
Phenobarbital	117, 175, 232, 260
Phenobarbital-D5	122, 180, 237, 265
Diphenylhydantoin	118, 194, 203, 280

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.

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.

9. **RECOMMENDED CAPILLARY COLUMNS** for adequate partitioning of all barbiturates would be 100% polydimethylsiloxane or 95% polydimethylsiloxane:5% phenyl