



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 6-ACETYLMORPHINE IN URINE BY GC/MS

May, 2011

Please see Notes and Supplemental Information before proceeding

SAMPLE PREPARATION

1. Add 4.0 mL of sample to a 16 x 100 mm disposable borosilicate glass culture tube.
2. Add appropriate amount internal standard. D-6 6-Acetylmorphine is preferred.
3. Add 1.0 mL of saturated sodium bisulfite and incubate at room temperature for 20 min.
4. Adjust pH to 2-3 with ≈ 0.7 mL of 10% HCL.

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 H₂O:0.25M Phosphate Buffer, pH 6.0. Prepare monthly. (Store refrigerated)
3. Wash column with 1.0 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. 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. Wash column with 3.0 mL of 0.1% HCl in deionized water.
3. Wash column with 1.0 mL of Methanol.
4. Wash column with 1.0 mL Ethyl Acetate.

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 1.5 mL of n-Butylchloride:Ethyl Acetate:TEA (76:20:04) . Made fresh daily.
3. Dry under N₂ or argon at 40°C. Over-drying may cause losses.

DERIVATIZATION - Using BSTFA

1. To each dried extract add 50 µL Acetonitrile and 50 µL BSTFA with 1% TMCS.
2. Mix the tube contents, cap and incubate at 70°C for 20 min.
3. Transfer to vials with inserts and cap.

Drug	Ions Monitored
6-Monoacetylmorphine	340, <u>399</u> , 400
6-Monoacetylmorphine-d3	290, <u>343</u> , <u>402</u>
6-Monoacetylmorphine-d6	290, 343, <u>405</u>

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.

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.
9. **RECOMMENDED CAPILLARY COLUMNS** for adequate partitioning are 5% phenyl, 35% phenyl or 100% polydimethylsiloxane,
10. **SATURATED SODIUM BISULFITE SOLUTION**
Dissolve 25 grams of Sodium Bisulfite (or Sodium Metabisulfite which converts to Sodium Bisulfite in solution) in 50 mL of Deionized water. Shake well. Dilute 1:1 with Phosphate Buffer, pH 6.0 for a total volume of 100 mL. Shake well. Prepare monthly and store reagent in a glass bottle with a Teflon or Polypropylene lined screw capped top.

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