



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 KETAMINE IN URINE BY GC/MS

SAMPLE PREPARATION - (Please see Notes and Supplemental Information before proceeding)

1. Add 1.0 mL of urine to a 16 x 100 mm disposable borosilicate glass tube with an inert screw cap.
2. Add 50 ng of Ketamine-d4 to each sample.
3. Add 3.0 mL of 0.25M Phosphate Buffer, pH 6.0.
4. If cloudy or precipitated centrifuge for 3 minutes at 3000 RPM.

Note: When adding an internal standard dissolved in an organic solvent to a urine or blood sample, the solvent volume must not exceed 3% of the buffered sample volume. Higher solvent concentrations may produce extraction losses.

HARDWARE SETUP - (Please refer to the Detectabuse Hardware Setup Instructions)

COLUMN CONDITIONING- (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. Allow to flow by gravity.
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. Proceed to Sample Extraction within 60 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. Allow to flow by gravity.
2. Wash with 1.0 mL of deionized water. Allow to flow by gravity.
3. Proceed to Sample Extraction within 60 min of column conditioning.

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(See: Column Conditioning – Revised GV-65C conditioning)

SAMPLE EXTRACTION - (Please see Note at end of this section before proceeding)

1. Pour samples onto preconditioned column. Allow to flow by gravity. Samples will flow through the column at a rate of 1-2 mL/min.
2. Wash column with 3.0 mL of deionized water. Allow to flow by gravity.
3. Wash column with 2.0 mL of Methanol. Allow to flow by gravity.
4. Wash column with 1.0 mL Ethyl Acetate. Allow to flow by gravity. Proceed to Sample Elution.

Note: If liquids do not elute freely by gravity flow, there is probably air trapped within the column bed or frits. Tapping the column mounting plate onto the vacuum box should initiate flow.

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 15 x 85 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-Butyl Chloride:Ethyl Acetate (80:20) with 4% Triethylamine (TEA)* to each column and allow solvent to flow through the columns by gravity into the test tubes.
4. Dry under N₂ or argon at less than 50°C.

* **Elution Solvent with 4% TEA** (4 mL TEA is added to 96 mL of n-Butyl Chloride:Ethyl Acetate, 80:20) is stable for approximately one week stored in a glass bottle with a Teflon or polypropylene lined cap. Close bottle tightly when not in use. A white residue begins to appear in the dried down eluate when the TEA begins to deteriorate. Artifacts from this process may interfere with "fast" GC/MS methods.

Note: If liquids do not elute freely by gravity flow, there is probably air trapped within the column bed or frits. Tapping the column mounting plate onto the vacuum box should initiate flow.

DERIVATIZATION (Only required if being assayed with Amphetamines)

Using HFBA:

1. Add 50 µL Ethyl Acetate and 50 µL Heptafluorobutyric Anhydride (HFBA) to each dried column eluate, cap tubes and gently mix the contents.
2. Heat at 65°C for 20 min.
3. Add 100 µL of Tartaric Acid** in Ethyl Acetate (1mg/mL) to each tube, followed by gentle mixing.
4. Dry down the eluates at less than 50°C and reconstitute with 100 µL Ethyl Acetate.
5. Inject 1 µL or transfer to an autosampler vial.

** This is a saturated solution. Allow the crystals to settle before pipetting 100 µL aliquots into the elution tubes.

WITHOUT DERIVATIZATION

1. Add 100 µL of Ethyl Acetate to each tube.
2. Inject 1 µL or transfer to an autosampler vial.

SUPPLEMENT - When using an automated robotic system

All liquids may be allowed to flow unassisted through the column or may be pulled through the column with vacuum or pushed through with positive pressure.

Assisted flow parameters may be set as follows:

Column Conditioning - Pass through column in approximately 20 seconds (± 20%).
Sample, Sample Washes, and Elution Solvent - Pass through column in approximately 60 seconds (± 20%).

GC/MS ANALYSIS

GC/MS: Hewlett-Packard equipped with Mass Selective Detector
GC Column: H.P. Ultra 2 Capillary Column (or equivalent), 15 m x 0.25 mm, 0.25 µm film thickness
Acquisition Mode: SIM
Temperature Program:
Injector Temp.: 175°C
Detector Temp.: 250°C
Initial: 75°C, Hold for 0.5 min., program at 20°C/min. to 205°C
Final: 275°C at 40°C/min.
Equil. Time: 1.0 min.
Split Ratio: Splitless
He Flow: 1.0 mL/min. @ 200°C
Septum Purge: 2 mL/min.
Purge Off Time: 1.0 min.
Solvent Delay: 4.0 min.
Dwell: 30
Start Acq.: 3.0 min.
Stop Run: 9.0 min.

MSD SIM PROGRAM

Underivatized Drug	Ions Monitored	Retention Time (min.)
Norketamine	166, 168, 195	6.91
Ketamine	180, 182, 209	7.12
Ketamine-d4	184, 186, 213	7.10

HFBA Derivatization Drug	Ions Monitored	Retention Time (min.)
Norketamine	340, 356, 384	6.80
Ketamine	210, 362, 370,	7.61
Ketamine-d4	366, 374	7.60

Retention time and ion spectra will vary somewhat from instrument to instrument.

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.