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DETECTABUSE® "NO VACUUM" GRAVITY SERIES GV-65 / GV-65C METHOD FOR THE ANALYSIS OF KETAMINE IN URINE BY GC/MS

JUNE 2010

Please see Notes and Supplemental Information before proceeding

SAMPLE PREPARATION

1. Add 1.0 mL of sample to a 16 x 100 mm disposable glass culture tube.
2. Add 50 ng of Ketamine-D4 to each sample.
3. Adjust pH to 2 with ≈ 0.1 – 0.2 mL of 10% HCl.
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. Proceed to Sample Extraction within 60 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 60 min. of column conditioning.

SAMPLE EXTRACTION

1. Pour samples onto preconditioned column.
2. Wash column with 3.0 mL of 0.01% HCL in deionized water.
3. Wash with 1.0 mL of Methanol.
4. Wash 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 (80:20) with 4% Triethylamine (TEA). Make fresh daily.
3. Dry under N2 or argon at less than 50°C. Over drying may cause losses.

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. Dry down the eluates at less than 50°C and reconstitute with 100 µL Ethyl Acetate.
4. Inject 1 µL or transfer to an autosampler vial.

WITHOUT DERIVATIZATION

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

MSD SIM PROGRAM

Underivatized

Drug	**Ions Monitored**
Norketamine	<u>166,</u> 168, 195
Ketamine	<u>180</u>, 182, 209
Ketamine-D4	184, 186, 213

HFBA Derivatization

Drug	**Ions Monitored**
Norketamine	340, <u>356</u>, 384
Ketamine	<u>210</u>, 362, 370,
Ketamine-D4	<u>366</u>, 374

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.

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.