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DETECTABUSE® "NO VACUUM" GRAVITY SERIES GV-65 / GV65C METHOD FOR THE ANALYSIS OF COCAINE AND COCAINE METABOLITES IN URINE BY GC/MS

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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 appropriate amount of internal standard. Benzoylecgonine D3 or D6 and/or Cocaine-D3.
3. Add 1.0 mL 1% HCl in deionized water. Sample must be between pH 2 and 3.

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 20 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. 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 0.01% HCL in DI H2O.
3. Wash with 0.5 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

1. To each dried extract add 50 µL Ethyl Acetate, vortex mix, then add 50 µL MTBSTFA with 1% TBDMCS <u>or</u> 50 µL BSTFA with 1% TMCS.
2. Mix the tube contents, cap and heat at 70°C for 20 min.
3. Transfer to vials with inserts and cap.

MSD SIM PROGRAM

BSTFA w/1% TMCS Drug	Ions Monitored
Benzoylecgonine	<u>240</u>, 256, 361
Benzoylecgonine-D3	<u>243</u>, 259, 364
Cocaine	<u>182</u>, 198, 303
Cocaine-D3	<u>185</u>, 201, 306
Cocaethylene	<u>196</u>, 272, 317
Cocaethylene-D3	<u>199,</u> 275, 320

MTBSTFA w/1% TBDMCS Drug	Ions Monitored
Benzoylecgonine	<u>282</u>, 346, 403
Benzoylecgonine-D3	<u>285</u>, 349, 406
Cocaine	<u>182,</u> 198, 303
Cocaine-D3	<u>185</u>, 201, 306
Cocaethylene	<u>196</u>, 272, 317
Cocaethylene-D3	<u>199</u>, 275, 320

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 of all amphetamines would be 100% polydimethylsiloxane, 5% phenyl or 35% phenyl columns.

10. **ADDITIONAL DERIVATIZING** include PFPA/PFPOH and DMF/DMFPA

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.