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

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Please see Notes and Supplemental Information before proceeding

SAMPLE PREPARATION

1. Add 5.0 mL of urine to a 16 x 100 mm disposable borosilicate glass tube with an inert screw cap.
2. Add 10 ng of Lysergic Acid Methylpropylamide (LAMPA) per mL of sample as internal standard. Mix.
3. Add 0.5 mL 10% HCl in deionized water. Mix.

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. 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 deionized water.
3. Wash column with 2.0 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 2.0 mL of n-Butyl Chloride with 4% Triethylamine (TEA)
3. Dry under N2 or argon at 50°C.

DERIVATIZATION

1. To each dried extract add 50 µL BSTFA and 15 µL TMS-imidazole.
3. Mix the tube contents, flush with nitrogen or argon and cap the tube or transfer contents into 100 µL reaction vials and seal.
4. Incubate the mixture @ 70°C for 20 min.
5. Allow the mixture to come to room temperature. Inject 2.0 µL.

MSD SIM PROGRAM

BSTFA/TMSI

Drug	Ions Monitored
2-Oxo-3-Hydroxy-LSD	307, <u>309</u>, 397
Iso-LSD	<u>279</u>, 293, 395
LSD	279, 293, <u>395</u>
LAMPA	279, 293, <u>395</u>

NOTES:

1. **<u>SAMPLES AND WASHES</u>** – Allow all samples and washes to gravity flow completely through the resin bed before adding the next liquid.

2. **<u>INTERNAL STANDARDS</u>** – 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. **<u>TURBID SAMPLES</u>** may need to be centrifuged.

4, **<u>RINSE SOLVENTS</u>** should be delivered to the top part of the column to better remove the aqueous.

5. **<u>ELUTION SOLVENTS</u>** 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. PRECAUTION Derivatized LSD (Lysergic Acid Diethylamide) is subject to loss by adsorption in the injection sleeve and on the capillary column as it ages. When sensitivity begins to drop, change the injector sleeve and cut 6-8 inches off the capillary column**.**

We suggest that you first run an unextracted standard at the LOQ level to make sure that the "system" is sensitive enough to determine picogram levels of LSD.

LSD will bind irreversibly to Silicic Acid on the glass elution tubes. TEA in the Elution Solvent selectively binds to the active sites of the glass surface and protects LSD from breakdown.

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.