



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 LSD AND METABOLITES IN URINE BY GC/MS

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

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.
4. The occasional cloudy or precipitated sample should be centrifuged 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 5% 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 Notes 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 the columns to flow by gravity.
3. Wash column with 2.0 mL of Methanol. Allow the columns to flow by gravity.
5. Add 1.0 mL Ethyl Acetate. Allow the columns to flow by gravity.

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 2.0 mL of n-Butyl Chloride 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) 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 a sample does not elute freely by gravity flow, there is probably air trapped within the column bed or frits. In most cases, tapping the column will initiate flow.

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.

PRECAUTION: Derivatized LSD is subject to loss by adsorption in the injection sleeve and on the capillary column as it ages. When sensitivity begins to drop, inject a few 5 µL aliquots of Silyl-8 GC column conditioning solution (Pierce Reagents) onto the capillary column with the oven set to 150°C and the purge set to off. If this does not restore sensitivity, changing the injector sleeve and if necessary, cutting 6-8 inches off the capillary column should restore it.

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.

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.: 285°C

Detector Temp.: 300°C

Initial: 160°C, program at 20°C/min. to 320°C

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.

Dwell: 100

Solvent Delay: 6.5 min.

Start Acq.: 6.5 min.

Stop Run: 9.0 min.

MSD SIM PROGRAM

BSTFA/TMSI

Drug	Ions Monitored	Retention Time (min.)
2-Oxo-3-Hydroxy-LSD	307, <u>309</u> , 397	8.10
Iso-LSD	<u>279</u> , 293, 395	8.22
LSD	279, 293, <u>395</u>	8.35
LAMPA	279, 293, <u>395</u>	8.47

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, it 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.