



Biochemical Diagnostics, Inc.

DETECTABUSE™ GRAVITY SERIES GV-65 METHOD FOR THE ANALYSIS OF PSILOCIN IN URINE BY GC-MS

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.

Revised: October, 2003

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

1. Add 3.0 mL of urine to a 16 x 100 mm disposable borosilicate glass tube with an inert screw cap top.
2. Add 50 ng of Psilocin-d10 to each sample.
3. Add approximately 5000 units of Beta-Glucuronidase, Sigma type L-2, from Patella Vulgata, to each sample.
4. Mix gently and incubate at 50°C for 2 1/2 hours or 37°C for 4 hours. Complete hydrolysis is also achieved in 16 hours at room temperature (15 - 30°C).
5. Add 3.0 mL of Carbonate/Bicarbonate Buffer, pH 9.2.
6. 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

1. Wash column with 1 mL of Methanol. Allow to flow by gravity.
2. Proceed to Sample Extraction within 30 min. of column 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 0.0625M phosphate buffer, pH 9.0. (Add 1 part 0.25M Phosphate Buffer to 3 parts water). Allow the columns to flow by gravity.

3. Dry the columns by applying vacuum adjusted to at least 7" Hg for 5 minutes (Test by momentarily placing the heel of hand over the column top. A strong pull should be felt through the column)

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. Any columns that have not emptied within 5 or 6 min. may be induced with a low vacuum from a small vacuum pump.*

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-Butylchloride:Ethyl Acetate (8:2) to each column and allow solvent to flow through the columns by gravity into the test tubes.

4. Add to each eluate 100 µL of saturated tartaric acid in Ethyl Acetate (1 mg/mL concentration)

5. Dry under N₂ or argon at less than 55°C.

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. If this does not do the job, use a rubber bulb to gently push a few drops of elution solvent and trapped air into the collection tube. Allow the remainder of solvent to flow by gravity.*

DERIVATIZATION

1. To each dried extract add 75 µL Acetonitrile, vortex mix, then add 25 µL MSTFA.

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.

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%).

Column Drying Steps - Use 12-15 PSI of positive pressure for 40 seconds or vacuum set at 15" Hg for

30 seconds (These drying parameters are for individual columns).

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

Acquisition Mode: SIM

Temperature Program:

Injector Temp.: 265°C

Detector Temp.: 285°C

Initial: 130°C, program at 20°C/min. to 240°C

Final: program at 30°C/min. to 280°C
Hold for 1 Minute

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:20

Solvent Delay: 4.5 min.

Start Acq.:4.5min.

Stop Run: 8.0 min.

References: F.Hasler, D.F. Bourquin, R.M. Brenneisen, T. Bar, and F.X. Vollenweider, Urinary excretion Profiles of Psilocin After Oral Administration of Psilocybin. J. Anal. Toxicol., **21** (1997)

MSD SIM PROGRAM

MSTFA

Drug	Ions Monitored	Retention Time
Psilocin-D10	66, 358	5.33
Psilocin	58, 290, 348	5.35



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