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

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Please see Notes and Supplemental Information before proceeding

SAMPLE PREPARATION

1. Add 3.0 mL of sample 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, form Patella Vulgata, to each sample.
4. Mix gently and incubate at 50°C for 2.5 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

COLUMN CONDITIONING - *ALL LIQUIDS FLOW BY GRAVITY*

Column Conditioning

1. Wash column with 1 mL of Methanol.
4. Proceed to Sample Extraction within 20 minutes of column conditioning.

SAMPLE EXTRACTION

1. Pour samples onto preconditioned column.
2. Wash column with 3.0 mL of 0.0625M Phosphate Buffer, pH 9.0
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).

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-Butylchloride:Ethyl Acetate (80:20)
3. Add 100 µL of saturated Tartaric Acid in Ethyl Acetate (1 mg/mL concentration).
3. Dry under N2 or argon at less than 55°C.

DERIVATIZATION

1. To each dried extract add 75 µL Acetonitrile
2. Vortex Mix
3. Add 25 µL MSTFA
4. Incubate the mixture @70°C for 20 minutes
5. Allow to Cool
6. Transfer to vials with inserts and cap.

MSD SIM PROGRAM Drug	**Ions Monitored**
Psilocin-D10	<u>66</u>, 358
Psilocin	<u>58</u>, 290, 348

NOTES:

1. **<u>0.0625M Phosphate Buffer, pH 9.0:</u>** to prepare solution add 1 part 0.25M Phosphate Buffer, pH 9.0 to 3 parts DI water.

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

3. **<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 3% of the final prepared sample.

4. **<u>TURBID SAMPLES</u>** may need to be centrifuged

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

6. **<u>POLAR SOLVENTS</u>** 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. **<u>AIR TRAPPED</u>** 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. **<u>IDEAL FRAGMENTS</u>** 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, your laboratory must validate it before it is used to report patient values. We would appreciate your comments on its performance and welcome your suggestions for improvements or enhancements.