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DETECTABUSE® " NO VACUUM" GRAVITY SERIES GV-65 / GV-65C METHOD FOR THE ANALYSIS OF METHADONE, EDDP AND METHAQUALONE IN URINE BY GC/MS

Please see Notes and Supplemental Information before proceeding

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SAMPLE PREPARATION

1. Add 1.0 mL of urine to a 16 x 100 mm disposable borosilicate glass tube with an inert screw cap. (Up to 3 mL may be used).
2. Add 150 ng/mL of the appropriate deuterated standard to each sample.
3. Add 1.0 mL of 1 % HCL in H2O per mL of urine. Urine pH should be less than 3.0.

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. Wash column with 1 mL deionized water.
4. Proceed to Sample Extraction within 30 minutes 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 column with 1 mL deionized water.
3. Proceed to Sample Extraction within 30 minutes of column conditioning.

SAMPLE EXTRACTION

1. Pour samples onto preconditioned column.
2. Wash column with 3.0 mL of 0.01% HCL in DI H20.
3. Wash column with 2.0 mL of Methanol.
4. Wash column with 1.0 mL Ethyl Acetate.
5. Proceed to Sample Elution.

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 16 x 100 mm culture tubes. Make sure that the hole pattern on the plate matches the hole pattern on the rack.
3. Add 2.0 mL of Ethyl Acetate with 4%Triethylamine (TEA) to each column.
4. Dry under N2 or argon at less than 50°C

RECONSTITUTION

1. Add 100 µL of n-Butyl Chloride:Ethyl Acetate (1:1) to each dried extract, vortex mix.
2. Flush with nitrogen or argon and cap the tube or transfer contents into 100 µL reaction vials and seal.
3. Inject 1-2 µL.

MSD SIM PROGRAM

Underivatived Drug	**Ions Monitored**
EDDP(Methadone Metabolite)	262, 276, <u>277</u>
Methadone	<u>72</u>, 91, 178
Methaqualone	233, <u>235</u>, 250

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>** may be made up in an aqueous matrix. When adding an internal standard dissolved in an organic solvent to a sample, 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. Prepare fresh daily.
6. **<u>ELUTION SOLVENTS</u>** with the TEA should be made fresh daily.
7. **<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.
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, 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.