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DETECTABUSE™ GRAVITY SERIES GV-65 METHOD FOR THE ANALYSIS OF METHADONE, EDDP, METHAQUALONE, PROPOXYPHENE AND NORPROPOXYPHENE IN URINE BY GC/MS

Revised: March 2003

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

1. Add 1.0 mL of urine to a 16 x 100 mm disposable borosilicate glass tube with an inert screw cap top.
2. Add 150 ng/mL of the appropriate deuterated standard to each sample.
3. Add 70 µL of 2N Sodium Hydroxide.
4. Mix and allow to react at room temperature for 15 min. (This step forms an internal amide derivative of Norpropoxyphene).
5. Add 2.0 mL of 0.25M Phosphate Buffer, pH 9.0 .
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 2.0 mL of water. Allow the columns to flow by gravity.
3. Add 1.0 mL Water:Methanol (60:40) to each column. Allow to flow by gravity.
4. 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-Butyl Chloride:Acetonitrile (70:30) 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.

Note: If a sample does not elute freely by gravity flow, there is either air trapped within the column bed or frits or aqueous phase remaining on the column because of weak vacuum during the column drying step. 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.

RECONSTITUTION

1. To each dried extract add 100 µL n-Butyl Chloride:Ethyl Acetate (1:1), vortex mix, then flush with nitrogen or argon.
2. Mix the tube contents, and cap the tube or transfer contents into 100 µL reaction vials and seal.
3. Inject 1-2 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 ($\pm 20\%$).

Sample, Sample Washes, and Elution Solvent - Pass through column in approximately 60 seconds ($\pm 20\%$).

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 thickness

Acquisition Mode: SIM
Injector Temp.: 270°C
Detector Temp.: 290°C
Temperature Program:
Level 1: 110°C, Hold for 0.5 min., program at 25°C/min. to 210°C
Level2: Program from 210°C-250°C at 10°C/min.
Level3: Program from 250°C- 280°C at 30°C/min.
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: 30
Solvent Delay: 3.5 min.
Start Acq.: 3.5 min.
Stop Run: 10.0 min.

MSD SIM PROGRAM

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Drug	Ions Monitored	Ret. Time (min.)
EDDP (Methadone Metabolite)	262, 276, <u>277</u>	5.70
Methadone	<u>72</u> , 91, 178	6.60
Methaqualone	233, <u>235</u> , 250	6.72
Propoxyphene	<u>58</u> , 91	6.82
Norpropoxyphene (Internal Amide)	91, 178, 197, <u>234</u>	8.20

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

