Biochemical Diagnostics, Inc.
An ISO 13485:2003 Company
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DETECTABUSE® MULTI-PREP® GVSA-200 METHOD FOR THE ANALYSIS OF ETHYL GLUCURONIDE (ETG) IN URINE BY LC-MS/MS

MAY 2013

Please see Notes and Supplemental Information before proceeding

SAMPLE PREPARATION-
1. Add 50 uL of sample to a disposable borosilicate glass tube.
2. Add 500 ng Etg internal standard /mL of sample (25 ng/50uL)
3. Add 950 uL acetonitrile

COLUMN CONDITIONING – *ALL LIQUIDS FLOW BY GRAVITY*

Column Conditioning and Activation of Cation Function using GVSA-200 Columns

1. Wash column with 1.0 mL methanol
2. Wash column with 1.0 mL deionized water
3. Wash column with 1.0 mL acetonitrile

SAMPLE EXTRACTION – *ALL LIQUIDS FLOW BY GRAVITY*

1. Pour pre-treated sample onto pre-conditioned column
2. Wash column with 1.0 mL deionized water
3. Wash column with 1.0 mL acetonitrile

SAMPLE ELUTION – *ALL LIQUIDS FLOW BY GRAVITY*

1. Place the column mounting plate on the elution rack loaded with corresponding labeled 12 X 75 or 13X100 mm tubes.
2. Make sure that the hole pattern on the plate matches the hole pattern on the rack.
3. Add 1 mL of acetonitrile: water: formic acid (95:4:1, v/v)

EVAPORATION
1. Evaporate to dryness

RECONSTITUTION
1. Reconstitute with 200 µL water/methanol (80:20, v/v) for analysis by LC-MS/MS

Notes:

1. **SAMPLES AND WASHES** – Allow all samples and washes to gravity flow completely through the resin bed before adding the next liquid.
2. **INTERNAL STANDARDS**– 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.
3. **TURBID SAMPLES** may need to be centrifuged.
4. **RINSE SOLVENTS** should be delivered to the top part of the column to better remove the aqueous.
5. **ELUTION SOLVENTS** should be made fresh daily.
6. **POLAR SOLVENTS** (e.g. acetonitrile and ethyl acetate) may absorb moisture. Flush bottles with nitrogen and keep stock bottles full to minimize moisture.
7. **AIR TRAPPED** 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. **IDEAL ION FRAGMENTS** should be determined by full scans of NEAT, 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.