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DETECTABUSE[®] GRAVITY SERIES GV-35C METHOD FOR BROAD SCREENING IN URINE OR ORAL FLUID USING LC/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.

Please see Notes and Supplemental Information before proceeding

SAMPLE PREPARATION - ALL LIQUIDS FLOW BY GRAVITY

SAMPLE HYDROLYSIS (if required)

1. Into a disposable borosilicate tube add 200 μL of 0.2M Acetate Buffer, pH 5.0 to each 200 μL sample aliquot. Verify pH 4.5 - 5.0.
2. Add an appropriate amount of the deuterated standard(s); which typically approximates the quantitation cutoff.
3. Add 1500 units of Beta-Glucuronidase, Helix Pomatia (or equivalent) per 200 μL of sample.
4. Mix gently and incubate at 55°C for two hours.
5. Add 350 μL of 0.25M Phosphate Buffer, pH 9.1 per 200 μL of sample. Final pH should be 8.5-9.0.
6. Centrifuge for 5 minutes at 3000 RPM.

SAMPLE PREPARATION WITHOUT HYDROLYSIS

1. Add the unhydrolyzed sample, or a measured aliquot, to a disposable borosilicate tube. The sample is adjusted to approximately pH 9.0 (as in step 5 above).
2. Add an appropriate amount of the deuterated standard(s); which often approximates the quantitation cutoff.

SPE COLUMN/ 96 WELL PLATE CONDITIONING - ALL LIQUIDS FLOW BY GRAVITY

1. Activate GV-35C resin with 1 mL of Methanol. Wait 2 minutes.
2. Wash GV-35C resin twice with 200 μL of 0.25M Phosphate Buffer, pH 9.1.
3. Proceed to Sample Extraction within 20 min. of column conditioning.

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SAMPLE EXTRACTION - ALL LIQUIDS FLOW BY GRAVITY

1. Transfer samples onto preconditioned columns or 96 well plate.
2. Wash with 0.5 mL 0.25M Phosphate Buffer, pH 9.1.
3. Dry the resin by applying positive pressure or, preferably, vacuum adjusted to at least 7" Hg for 3-5 minutes.

SAMPLE ELUTION - ALL LIQUIDS FLOW BY GRAVITY

1. Elute into a borosilicate glass tube or 96 well collection plate with 400 μL of Ethyl Acetate:Isopropanol (85:15) and allow to flow through by gravity.
2. Add an additional 400 μL of Ethyl Acetate:Isopropanol (85:15) and allow to flow through by gravity.
3. Following elution the eluate must be completely dried down under Nitrogen or Argon and reconstituted with a diluted solution of methanol compatible with the mobile phase used for LC/MS analysis.

NOTES:

1. *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.*
2. *When using a manual or automated system all liquids may be allowed to flow unassisted through the column. Ordinarily, after 2 minutes all liquids will have passed through the resin by gravity flow, however, any liquid remaining on the resin may be gently pulled through the column with vacuum or pushed through with positive pressure.*