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MULTI-PREP® "NO VACUUM" GRAVITY SERIES GVSA-200 METHOD FOR THE ANALYSIS METHYLMELONIC ACID IN SERUM OR URINE BY GC/MS

Please see Notes and Supplemental Information before proceeding

January 2011

SAMPLE PREPARATION:

1. 500 µL sample and/or control plus 50 µL of a 50 nano moles MMA- D3 /ml (in ethanol) internal standard is added to 13x100 mm test tubes.
2. 1 mL of 0.001M NaOH in water is added to each tube.
3. All tubes are vortex mixed.

COLUMN CONDITIONING - ALL LIQUIDS FLOW BY GRAVITY

1. Wash column with 1.0 mL of Methanol
2. Wash column with 1.0 mL DI H2O

HARDWARE SETUP - (Please refer to the Detectabuse Hardware Setup Instructions)

STANDARD PREPARATION:

1. A standard curve of MMA is prepared in 13x100mm test tubes as follows:

Nano Moles/L	Volume to Pipet
-0-	-0-
-50-	50 uL of 1000 nM/L
-100-	100 uL of 1000 nM/L
-500-	50 uL of 10000 nM/L
-1000-	100 uL of 10000 nM/L
-2000-	200 uL of 10000 nM/L
* 1.18mg/L = 10,000 nM/L	

2. 50 µL of a 50 nano moles MMA- D3 /ml (in ethanol) internal standard is added to each tube.
3. Dry at 60° - 70° C under Nitrogen or Argon.

SAMPLE EXTRACTION AND PURIFICATION:

1. Pour prepared samples onto the columns and allow them to drain by gravity flow.
2. Rinse emptied tubes with 1 mL DI H2O and transfer wash onto column.
3. Wash each column 2 mL of 0.001M NaOH in DI H2O.
4. Wash each column with 2 mL of 0.6 M Acetic Acid in Methanol.

SAMPLE ELUTION

1. Elute with 2.0 mL 5M Acetic Acid in Methanol into screw cap tubes with Teflon lined cap.
2. Dry at 60° - 70° C under Nitrogen or Argon. Remove from heat as soon as eluates have dried.

DERIVATIZATION

1. Add 75 uL Acetonitrile and 25 uL MTBSTFA to each dried eluate and vortex mix.
2. Incubate at 70° C for 30 minutes.
3. Vortex mix for 30 seconds followed by an additional 5 minutes of sonication or vortex mixing.

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
Acquisition Mode: SIM
Temperature Program:
Injector Temp.: 250°C
Detector Temp.: 300°C
Initial: 100°C, hold for 1 min., program at 15°C/min. to 175°C
Equil. Time: 1.0 min.
Split Ratio: Splitless
He Flow: 1.0 mL/min. @ 200°C
Septum Purge: 2 mL/min.
Purge Off Time: 1.5 min.
Dwell: 30
Solvent Delay: 3.0 min.
Start Acq.:3.0 min.
Stop Run: 10.0 min.

MSD SIM PROGRAM		
Drug	**Ions Monitored**	**Retention Time**
Methylmalonic Acid	289	5.76
Methylmalonic Acid – D3	292	5.75

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, 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.

NOTES:

1. **SAMPLES AND WASHES** – Allow all samples and washes to gravity flow completely through the resin bed before adding the next liquid.

2. SAMPLE EXTRACTION- 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.

3. 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. If prepared in an organic solvent the solvent must not exceed 5% of the final prepared sample.

4. TURBID SAMPLES may need to be centrifuged

5. RINSE SOLVENTS should be delivered to the top part of the column to better remove the aqueous.

6. ELUTION SOLVENTS with TEA should be made fresh daily.

7. POLAR SOLVENTS 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. 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.

9. IDEAL FRAGMENTS should be determined by full scans of neat, derivatized standards.

10. ROBOTIC SYSTEMS- 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 (± 20%).
Sample, Sample Washes, and Elution Solvent - Pass through column in approximately 60 seconds (± 20%).