



# Biochemical Diagnostics, Inc.

180 Heartland Blvd, Edgewood, NY 11717 • Phone (800) 223-4835

Fax (631) 595-9204 • www.biochemicaldiagnostics.com

## MULTI-PREP® GRAVITY SERIES GVSA-200 METHOD FOR THE ANALYSIS OF METHYLMALONIC ACID IN SERUM OR URINE USING GC/MS

Revised: May 2003

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

### 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 0.001M NaOH in H<sub>2</sub>O is added to each tube.
3. All tubes are vortex mixed.

### UNEXTRACTED 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 10,000 NM/L*
1000	100 uL of 10,000 NM/L
2000	200 uL of 10,000 NM/L

\* 1.18 mg/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° under argon or nitrogen.

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

### COLUMN PREPARATION:

Wash each column sequentially with the following solutions, allowing each wash to drain through the column by gravity flow.

1. 1 Methanol
2. 1 mL H<sub>2</sub>O

**SAMPLE EXTRACTION** - (Please see Notes at end of this section before proceeding)

### SAMPLE EXTRACTION AND PURIFICATION:

1. Pour prepared samples onto the columns and allow them to drain by gravity flow.
2. Rinse empty tubes with 1 mL H<sub>2</sub>O and transfer wash onto column.
3. Wash each column 2 mL of 0.001M NaOH in H<sub>2</sub>O.
4. Wash each column with 2 mL of 0.6 M Acetic Acid in Methanol.

**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.

### 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 dried unextracted standards and each dried eluate. 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.

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

**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****MTBSTFA**

Drug	Retention Time	Ions Monitored
Methylmalonic Acid	5.76	289
Methylmalonic Acid-D3	5.75	292

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.*