



## **METHYLMALONIC ACID FROM SERUM OR PLASMA FOR GC/MS ANALYSIS USING: 500 mg CLEAN-UP<sup>®</sup> QAX EXTRACTION COLUMN**

Part #:

CUQAX15Z – CLEAN-UP<sup>®</sup> QAX 500 mg, 10mL Tube

SMSTFA-1-1 – SELETRA-SIL<sup>®</sup> MSTFA w/ 1% TMCS

### **1. PREPARE SAMPLE:**

Add 100  $\mu$ L of internal standard D<sub>3</sub>-MMA and 1 mL of acetonitrile to 1 mL of plasma or serum.

Vortex for 20 sec.

Centrifuge for 5 min at 2000 rpm.

### **2. CONDITION CLEAN-UP<sup>®</sup> EXTRACTION COLUMN:**

1 x 3 mL CH<sub>3</sub>OH.

1 x 3 mL D.I. H<sub>2</sub>O.

### **3. APPLY SAMPLE:**

Decant supernatant onto SPE column.

### **4. WASH COLUMN:**

1 x 10 mL of D.I. H<sub>2</sub>O.

Dry with vacuum for 3 min.

1 x 5 mL of CH<sub>3</sub>OH.

Dry with vacuum for 3 min.

1 x 2 mL of methyl-tert-butyl ether (MTBE).

Dry with vacuum for 3 min.

### **5. ELUTE METHYLMALONIC ACID:**

1 x 5 mL of 3% Formic Acid in MTBE; collect at 1 to 2 mL/min.

### **6. DRY ELUATE:**

Dry under a stream of nitrogen at < 35 °C.

### **7. DERIVATIZE:**

Reconstitute with 25  $\mu$ L of MSTFA + 1% TMCS and 25  $\mu$ L Ethyl Acetate.

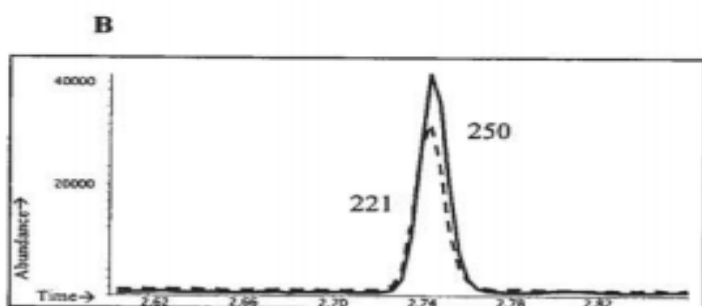
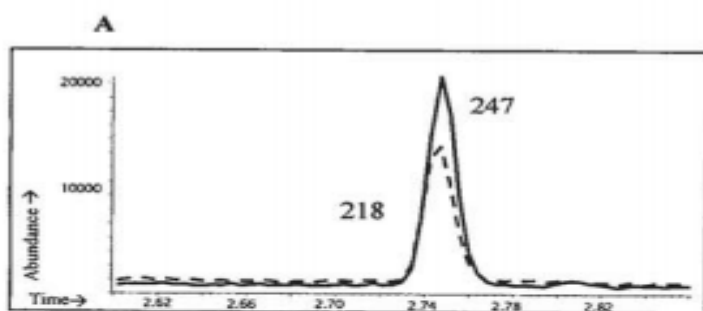
Heat for 20 min at 60 °C.

### **8. QUANTITATE:**

Inject 1 to 2  $\mu$ L onto gas chromatograph.

## INSTRUMENT CONDITIONS (GC-MS):

### CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion	Relative Retention Time (minutes)
1. Methylmalonic Acid	247	218	2.76
2. Methylmalonic Acid-D <sub>3</sub>	250	221	2.74

### PARAMETERS

**GC/MS:** HP 5890 w/ 5970 MS Detector with 7673 ALS System

**GC capillary column:** RtxR-200 MS 20m x 0.18mm, 0.4 $\mu$ m

**Injector:** 1 $\mu$ L Split 1:20 270 $^{\circ}$ C

**Oven temperature program:** 100 $^{\circ}$ C for 0.5min; 18 $^{\circ}$ C/min to 160 $^{\circ}$ C; 50 $^{\circ}$ C/min to 300 $^{\circ}$ C for 2.50minutes

**Carrier gas:** Helium

**MSD condition:** Aux temperature: 280  $^{\circ}$ C, MS Source: 250  $^{\circ}$ C, MS Quad: 150  $^{\circ}$ C

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