Simple Sample Preparation for Measuring Methylmalonic Acid in Blood Serum by LC-APCI-MS/MS.

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Many researchers need to measure methylmalonic acid (MMA) in blood serum to assess vitamin B12 sufficiency. Most perform sample preparations that involve laborious and expensive solid-phase or liquid extraction followed by derivatization to form the butyl ester. Butyl-MMA can then be measured by LC-MS or GC-MS. We developed a simpler approach to sample preparation similar to that of Turgeon, et al (1), which was used to measure MMA, homocysteine and methylcitric acid in dried blood spots. Our method involved protein precipitation of blood serum with acetonitrile containing the internal standard methyl-D3-malonic acid (MMA-D3 IS) followed by evaporation of the supernatant under stream of nitrogen at 40ºC for 15 minutes. After the residue was butylated by a mixture of acetyl chloride (10%) in 1-butanol for 15 minutes at 65ºC, the remaining butylation reagent was evaporated and the residue was reconstituted with a water/methanol (1:1) diluent. After transferring the diluent to an autosampler vial, a 20 µL injection was made into a solid-core C8, 2.6um, 150 x 2.1mm HPLC column heated to 50ºC. A 2-step gradient from 55% to 70% methanol in water containing 0.1% formic acid separated MMA from succinic acid (SA) and eluted them to an atmospheric-pressure chemical ionization (APCI) source of a tandem mass spectrometer (MS/MS). For quantitation, we monitored the positive-ion transitions *m/z* 231.1 to *m/z* 119.2 for MMA and *m/z* 234.1 to *m/z* 122.0 for the MMA-D3 IS. The MS/MS acquisition method included a polarity switch to negative ionization to clean the corona discharge needle (2) after analytes were eluted.

Although SA did not significantly affect MMA peak areas from the quantitation transition *m/z* 231.1 to *m/z* 119.2, it did when monitoring the confirming product ion m/z *101.2*, resulting in inconsistent ion ratios among various specimens. Since the simplified sample preparation did not attempt to reduce interference from SA, ion-ratio conformation could not be used. However, the

method reliably identified specimens that had less than 360 nM of MMA, indicative of vitamin B12 deficiency. The variation of MMA concentrations measured in quality controls (QCs) within batches was 6% CV (n = 20) and between batches was less than 7% CV (n = 20 across 4 days).

Our five-minute LC-MS/MS method reliably achieved a quantitative range of 50 to 1000 nM with a throughput of 12 samples per hour on a single channel of a multi-channel HPLC system.

1. Turgeon CT, Magera MJ, Cuthbert CD, Loken PR, Gavrilov DK, Tortorelli S, Raymond KM, Oglesbee D, Rinaldo P, and Matern D. Determination of total homocysteine, methylmalonic acid, and 2-methylcitric acid in dried blood spots by tandem mass spectrometry. Clin Chem 2010; 56:1686–1695.

2. Di Bussolo, JM. Method for cleaning an atmospheric pressure chemical ionization source. Thermo Finnigan LLC (San Jose, CA) patent pending (docket 14649US1/NAT), March 2014.