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Simple and Rapid Tandem Mass Spectrometry Method for the Analysis of Methylmalonic Acid in Urine

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Introduction[1]

- Vitamin B12 deficiency can lead to irreversible neurological symptoms (e.g. memory deficits, gait ataxia)
- Up to 40% of older adults show metabolic signs of B12 deficiency
- Up to 56% of adults with metabolic signs of B12 deficiency present normal levels of serum B12
- B12 deficiency causes increased levels of serum and urine methylmalonic acid (MMA), a specific biomarker

Objectives

- To develop a multiplex UPLC-MS/MS method for the analysis of MMA and creatinine in urine (non-invasive specimen collection)
- To determine MMA/creatinine levels in a group of 35 older adults from the NuAge study (>70 yrs) at different stages of metabolic B12 deficiency
- To compare the MMA results obtained using this method with those obtained with a GC/MS validated method necessitating derivatization
- To perform correlation studies between urine and plasma MMA levels
- To analyze MMA/creatinine ratios in urine samples collected on filter paper from newborns having B12 deficiency

Method

Urine sample analysis by UPLC-MS/MS

- 30 µL of urine mixed with 60 µL of water containing MMA-D_3 and creatinine-D3 as internal standards
- Calibration curves
  - MMA: 0 to 500 µM
  - Creatinine: 0 to 30 mM
- Acquity I-Class UPLC (Waters)
  - Column Acquity CSH C18, 1.7 µm, 2.1 x 50 mm (Waters)
  - Phase A = MeOH, 0.1% formic acid
  - Phase B = H_2O, 0.1% formic acid
  - Gradient: 0 → 0.5 min, 100% B
    0.5 → 1.0 min, 100 → 90% B
    1.0 → 1.3 min, 90% B
    1.3 → 1.8 min, 90% → 10% B
    1.8 → 2.3 min, 100% B
- Xevo TQ-S micro mass spectrometer (Waters)
  - Multiple reaction monitoring
  - ESI+: Creatinine (+1): *m/z* 115.07 → 45.05
    Creatinine-D_3: *m/z* 117.09 → 47.07
    ESI-: MMA: *m/z* 117.02 → 73.03
    MMA-D_3: *m/z* 120.04 → 76.05

Waters Corp.

Urine and plasma sample analysis by GC-MS

- Method based on the following references[2,3]

Results

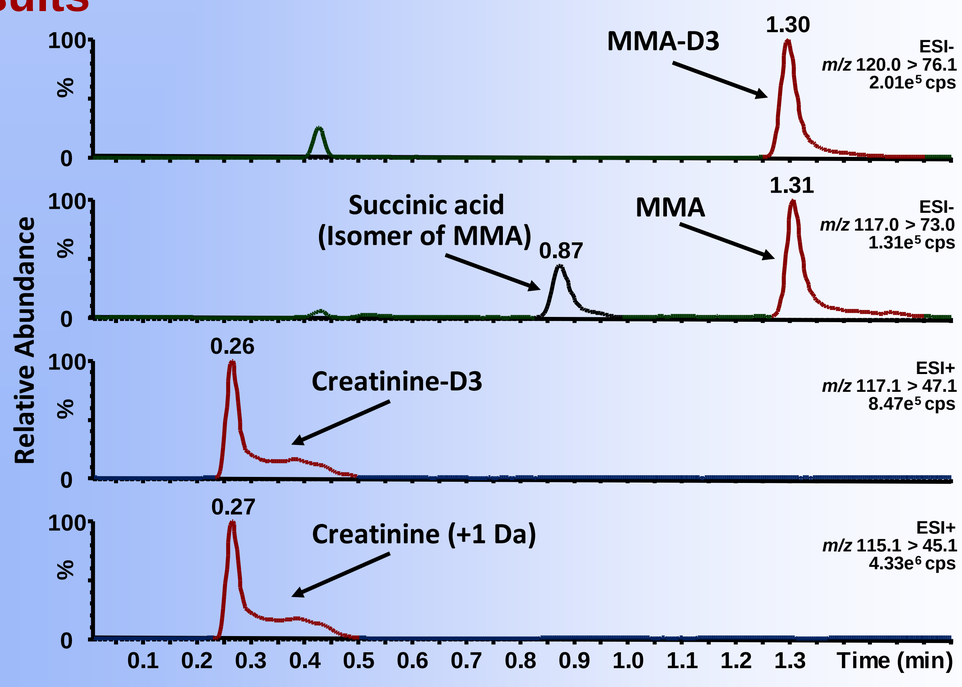

Figure 1: UPLC-MS/MS ion chromatograms of MMA and creatinine in urine

Results

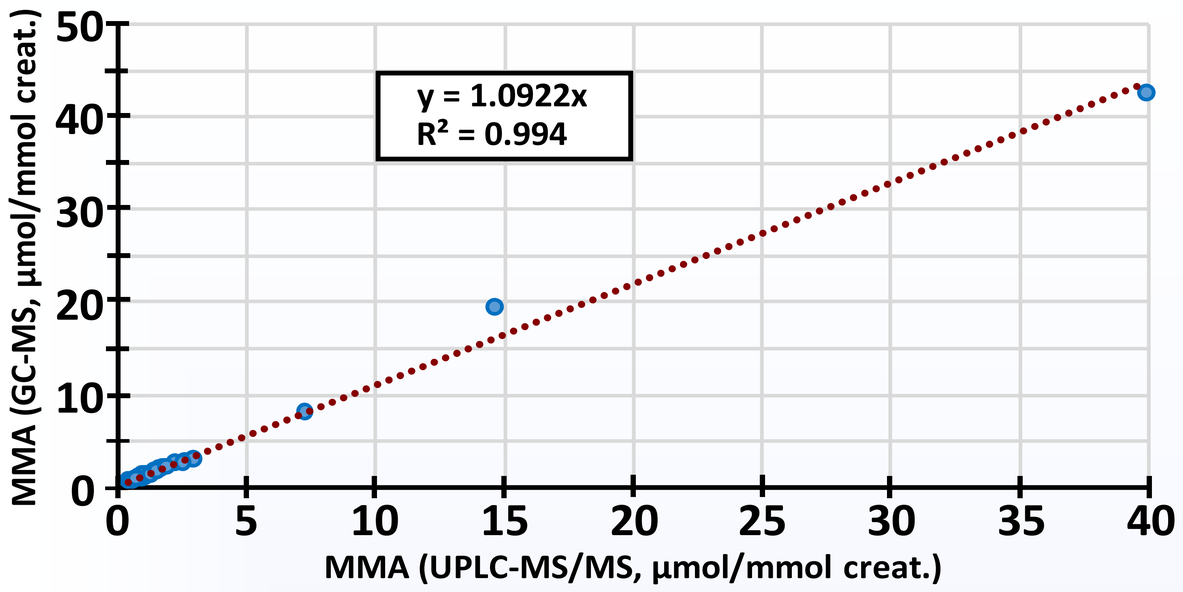

Figure 2: Comparison of MMA levels measured in urine from 35 older adults by UPLC-MS/MS and GC-MS

The results obtained with the new UPLC-MS/MS method correlate well with the results obtained with the validated GC-MS method

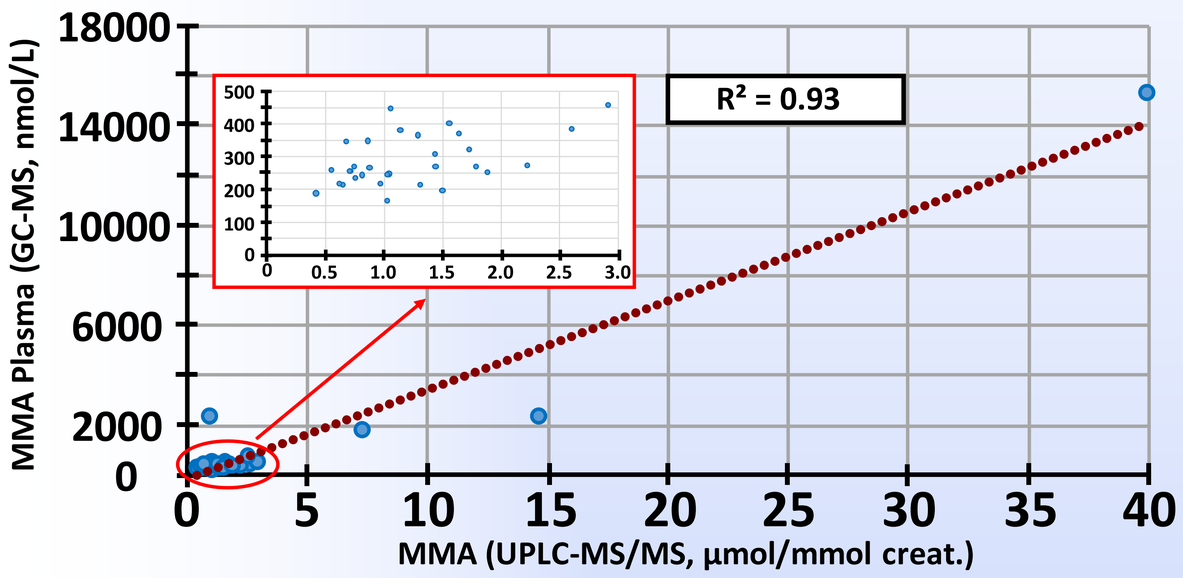

Figure 3: Comparison of MMA levels measured in urine and in plasma for 35 older adults by UPLC-MS/MS

The correlations between MMA results obtained in urine and plasma for the same patients were significant (Spearman r = 0.59)

Newborn urine screening

MMA was analyzed in dried urine filter paper samples from 4 newborn babies collected at 21 days of life, presenting vitamin B12 deficiency. MMA concentration levels ranged from 114 to 3402 µmol/mMol creatinine

Conclusions

- A simple and rapid method for the multiplex analysis of urinary MMA and creatinine by UPLC-MS/MS was developed
- The results of the new UPLC-MS/MS method for the analysis of MMA correlate well with the results obtained with a GC-MS validated method
- The MMA levels measured in urine showed significantly positive correlations with the levels measured in plasma
- The MMA concentration was measured in urine from 35 older adults at different stages of metabolic B12 deficiency and in dried urine spots of newborn babies with a B12 deficiency

Acknowledgements

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References:

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