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A novel mass spectrometry kit for MMA in plasma/serum and urine

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Introduction

Vitamin B_{12} is a water-soluble vitamin with a key role in the vital functioning of the brain and nervous system, as well as in the formation of blood. Furthermore, it is also involved in cell metabolism, energy production, fatty acid synthesis, DNA synthesis and regulation. Vitamin B_{12} deficiency is common in the general population, particularly in elderly people and vegetarians [1]. It causes hematologic and/or neuropsychiatric diseases and increases the risk of age-associated disorders like dementia, depression and bone fractures. The vitamin B_{12} deficiency-related neurological sequela are mostly non-specific, but can cause irreversible damage if not treated rapidly. The specific and sensitive diagnosis of vitamin B_{12} deficiency is therefore absolutely essential. The metabolic marker methylmalonic acid (MMA), which increases in plasma and urine in the case of vitamin B_{12} deficiency, fulfils both the sensitivity and specificity criteria [2]. By analysing plasma MMA levels, it is possible to distinguish persons according to their dietary habits (vegetarians, omnivores etc.) to diagnose vitamin B_{12} deficiency in Metformin users, a diabetic drug, and to optimise the success of vitamin B_{12} supplementations. However, until now, MMA levels have mainly been measured with GC-MS methods, which can be expensive and are often not available in modern laboratories.

Chromsystems GmbH has developed a reagent kit for the specific determination of MMA in plasma/serum and urine using LC-MS/MS with matrix-specific controls and calibrators. The aim of this study was to evaluate this new kit in comparison to GC-MS as a scientifically recognised reference method using plasma and urine samples.

Methods

In this study, the novel kit was compared with the reference GC-MS method using plasma (n = 131) and urine (n = 126) samples. Sample preparation for serum/plasma involves the use of clean-up tubes, allowing fast sample preparation and clear eluates for direct injection into an LC-MS/MS system. For urine samples, a similar "dilute and shoot" procedure without further preparation steps was used. Other vitamin B_{12}-related markers were measured in blood to validate the diagnostic power of this new LC-MS/MS method.

MassChrom® Methylmalonic Acid in Plasma/Serum and Urine, reagent kit for LC-MS/MS analysis (Chromsystems, Gräfelfing/Germany)

For plasma/serum 100 µL patient samples/calibrators/controls were added in clean-up tubes and vortexed with 50 µL internal standard. Afterwards, the solutions were centrifuged for 10 min at 14000 x g. The filtrates were transferred into autosampler vials. 10 to 20 µL of the filtrates were injected into the LC-MS/MS system (ABSciex 4500). For urine samples 200 µL patient samples/calibrators/controls were mixed (vortex) with 25 µL internal standard for 10 s. The samples were diluted with dilution buffer and transferred into autosampler vials. 10 to 20 µL were injected into the LC-MS/MS system (ABSciex 4500).

GC-MS reference method

The GC-MS reference method was performed as described elsewhere [3]. Briefly, sample mixture consisting of plasma/serum or urine, water and internal standard (MMA-D4) were transferred to an anion exchange chromatography column and after several washing steps with water and acidic acid in methanol MMA was eluted using a solution of acidic acid and HCl from the resins. Finally, MMA derivatisation was performed with MTBDSFA in acetonitrile and measured by GC-MS.

Results

The results of the sample measurements after sample preparation using the commercial kit compared with values obtained with the GC-MS method revealed linear regression curves y = 0.941x + 41.681 (r^2 = 0.994) in plasma and y = 0.958x + 0.386 (r^2 = 0.980) in urine, indicating good agreement between both methods. Furthermore, the results from plasma/sample determination by the reference method or LC-MS/MS also showed a strong correlation and excellent agreement with other vitamin B_{12} markers (active B_{12}, total homocysteine; results not shown).

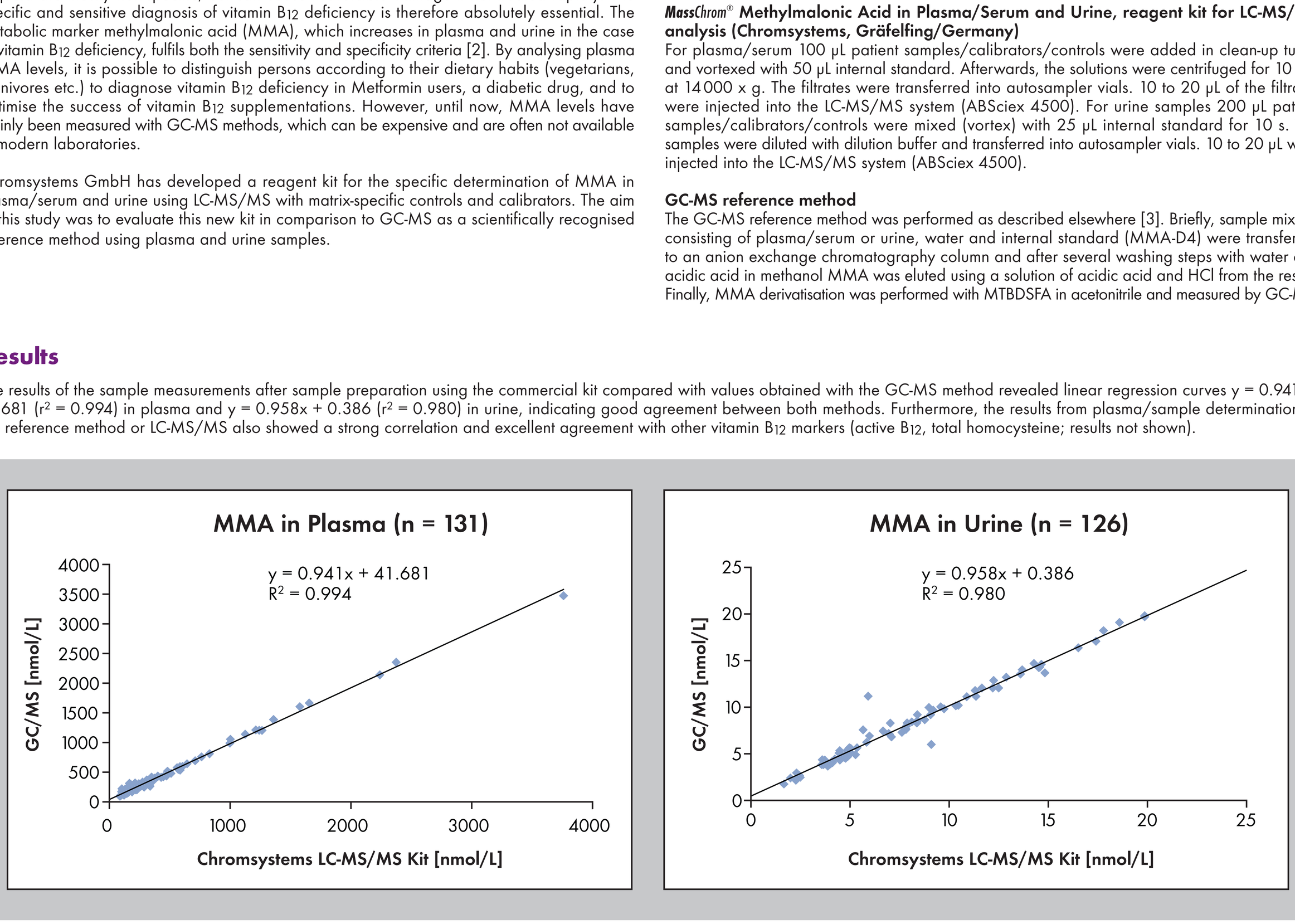

Figure 1: Comparison of Chromsystems LC-MS/MS method vs. GC-MS reference method for the determination of MMA in plasma (n = 131) and urine (n = 126).

Conclusion

This novel assay for the determination of MMA from plasma/serum and urine using LC-MS/MS is comparable with the GC-MS reference method in accuracy. The method is characterised by fast sample preparation, minimal matrix effects, high precision, robustness and cost-effectiveness.

References

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