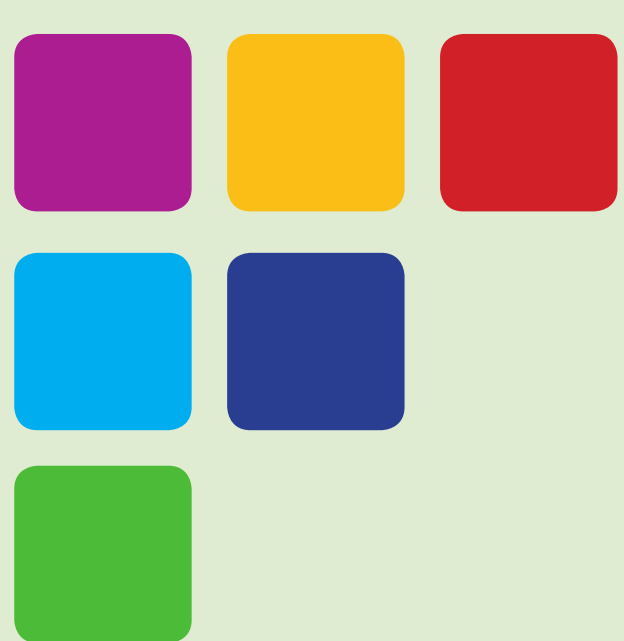




Comparison of three different calibrators for the analysis of vitamin B1 and B6 using LC-MS/MS

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Background

Different laboratory tests are available to determine vitamin B1 and B6 status. Recently, liquid chromatography coupled to tandem mass spectrometry (LC-MS) has gained much interest due to its simplified sample preparation. Different commercial parties offer B1/B6 calibrators for different matrices. Here, we compared different calibrators in a new LC-MS test for the analysis of vitamin B1 and B6 in whole blood

Methods

- Comparison of three calibrators: lyophilized whole blood (C1), standard addition whole blood (C2), and lyophilized serum protein (C3);
- Analysis of patient samples, SKML controls and C1-C2-C3;
- Protein precipitation of whole blood sample (50 μ L) with 20% trichloroacetic acid (TCA) using lyophilized whole blood calibrators;
- LC-MS analysis of supernatant on Waters UPLC I-class coupled to a Xevo TQ-S micro triple quadrupole MS.
- Additional method comparison against HPLC method (lyophilized whole blood calibrator) and external lab LC-MS method (protein matrix calibrator).

Results

- Calculated result for vitamin B1 depended on the selected calibrator (Figure 1A). Results calculated with C2 were 30-40% lower than those calculated with C1 and C3;
- Calculated results for vitamin B6 differed < 20% between the different calibrators (Figure 1B);
- Deming regression slopes for comparison HPLC-LCMS: 1.071 for B1 (Figure 2A) and 0.941 for B6 (Figure 2B);
- Deming regression slopes for comparison LCMS-external lab LCMS: 1.307 for B1 (Figure 2C) and 0.962 for B6 (Figure 2D).

Conclusions

The choice of calibrator will greatly affect the calculated patient result, which is especially apparent for vitamin B1. More work is needed to determine the cause of the discrepancies and to improve harmonization between methods.

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Figure 1A

Comparison of different vitamin B1 calibrators

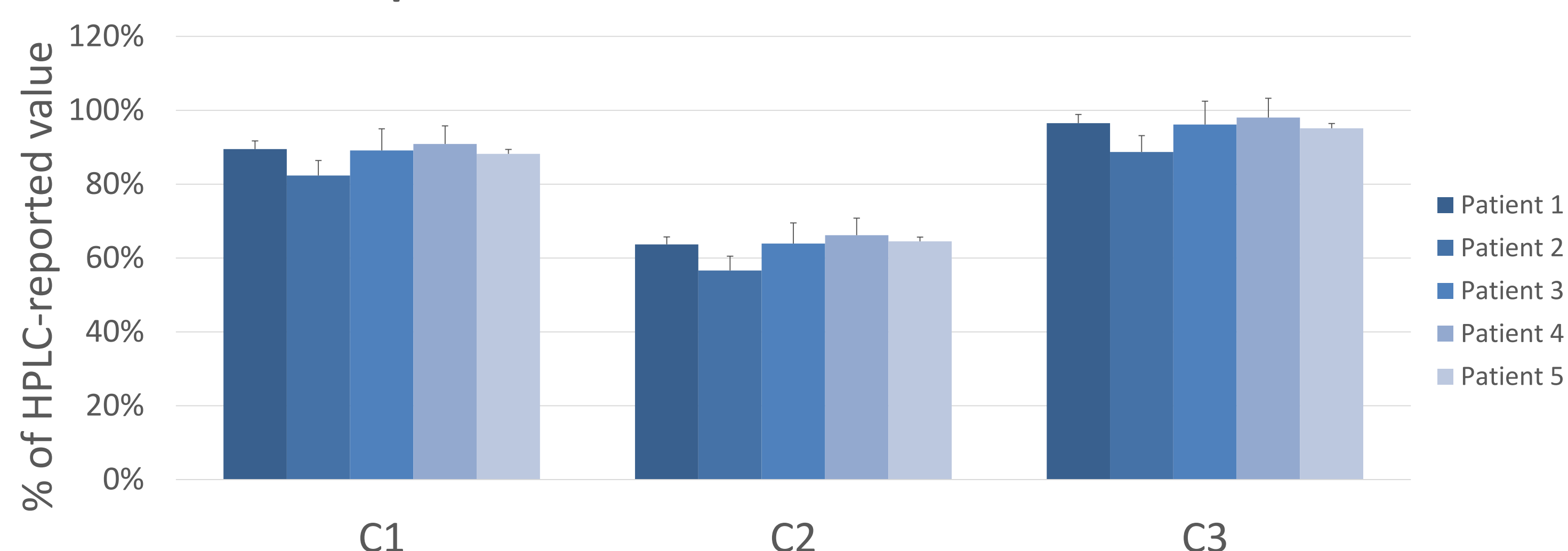


Figure 1B

Comparison of different vitamin B6 calibrators

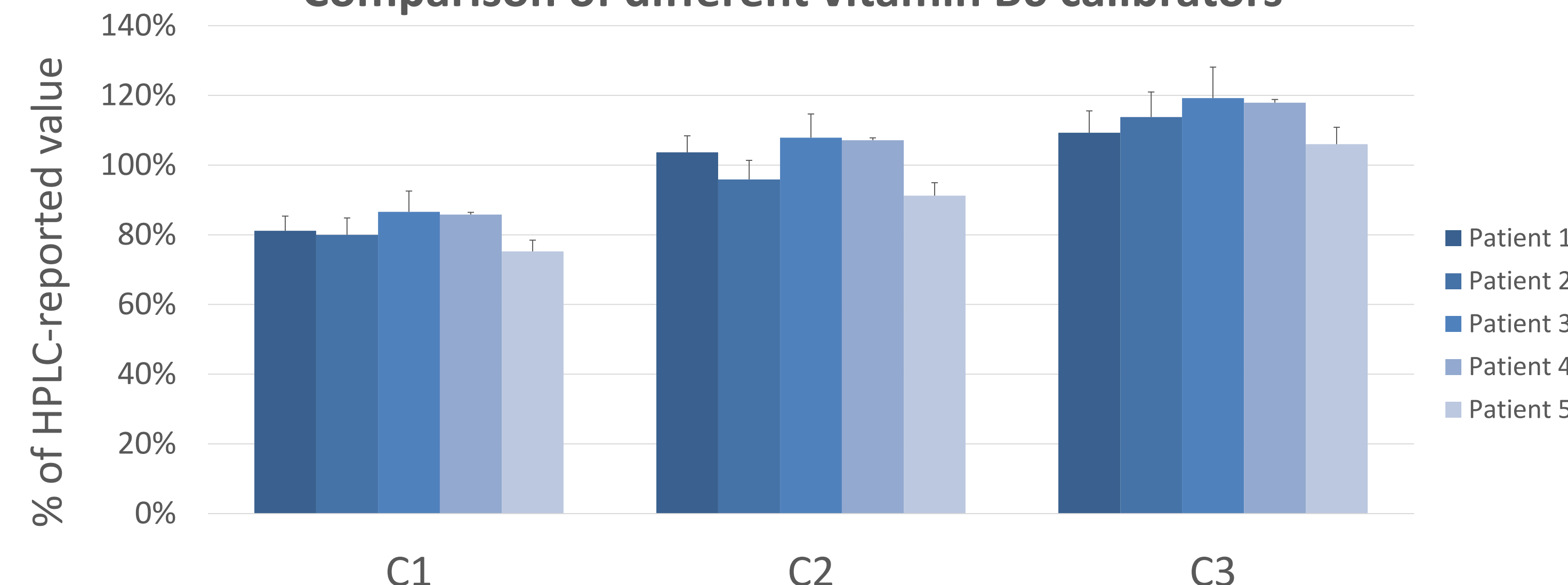


Figure 1: comparison of three calibrators for the analysis of vitamin B1 (A) and B6 (B). Five patient samples were first analyzed using an existing HPLC method, and subsequently on LC-MS. Results are expressed as % of the HPLC result.

Figure 2A

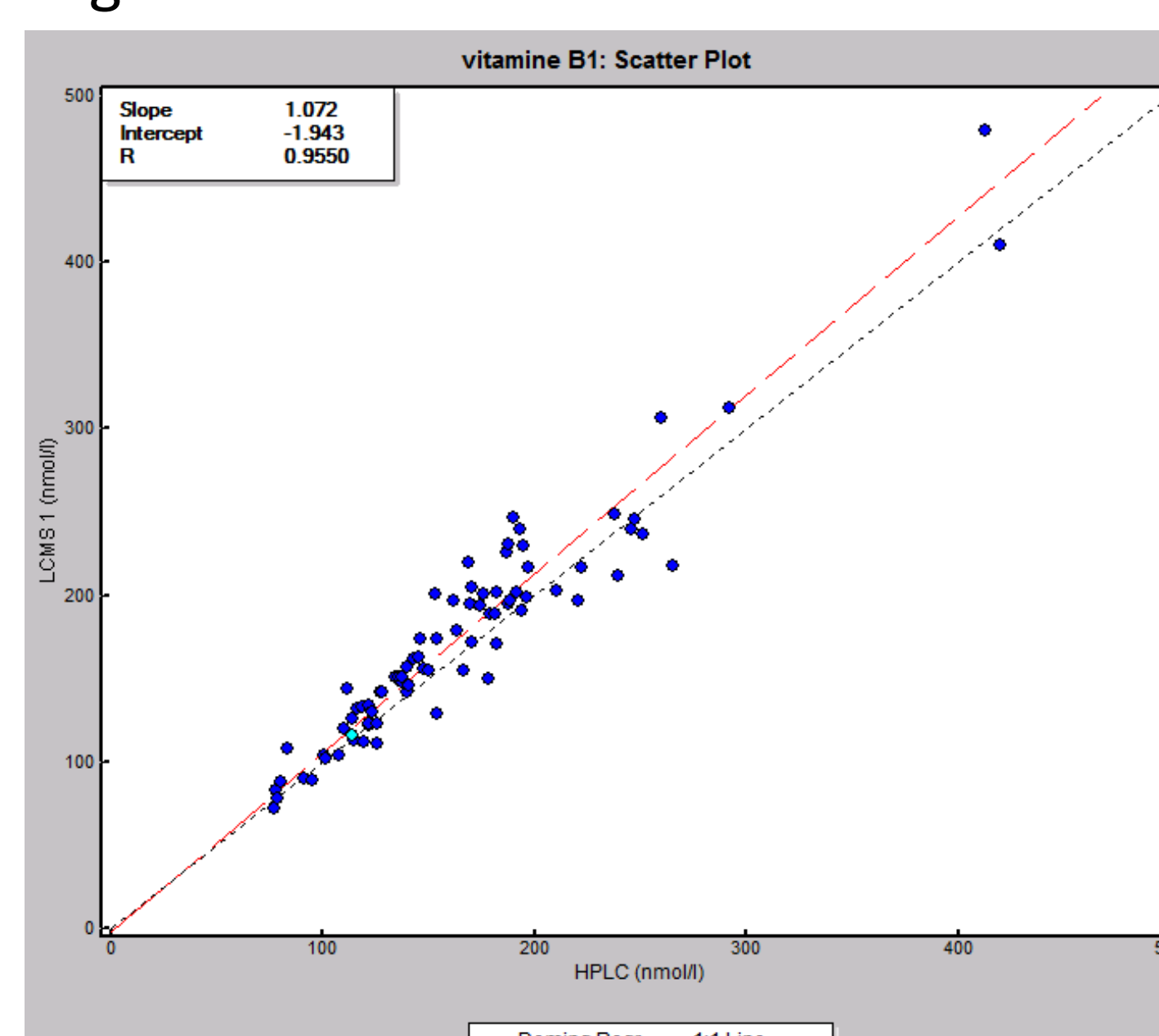


Figure 2B

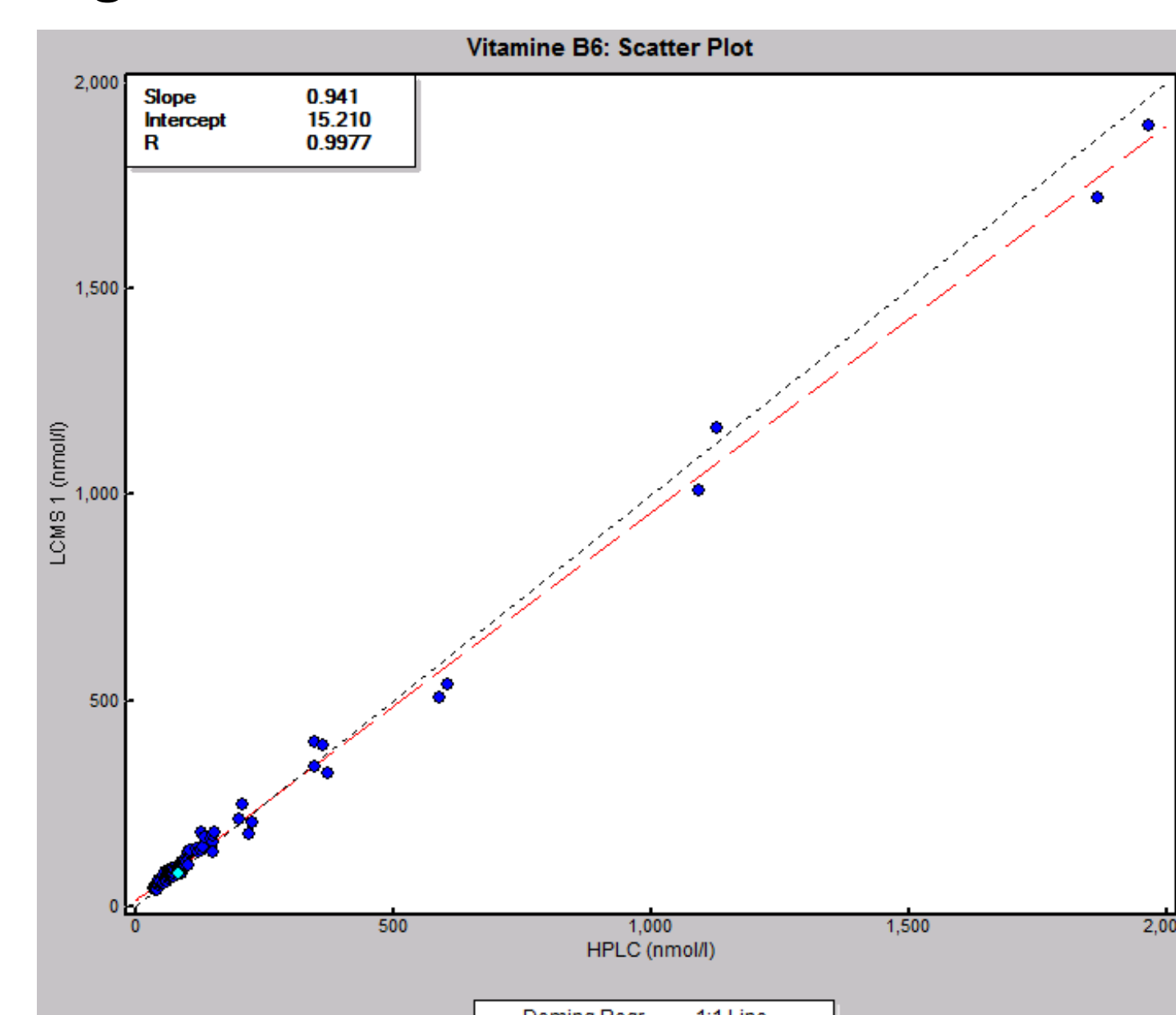


Figure 2C

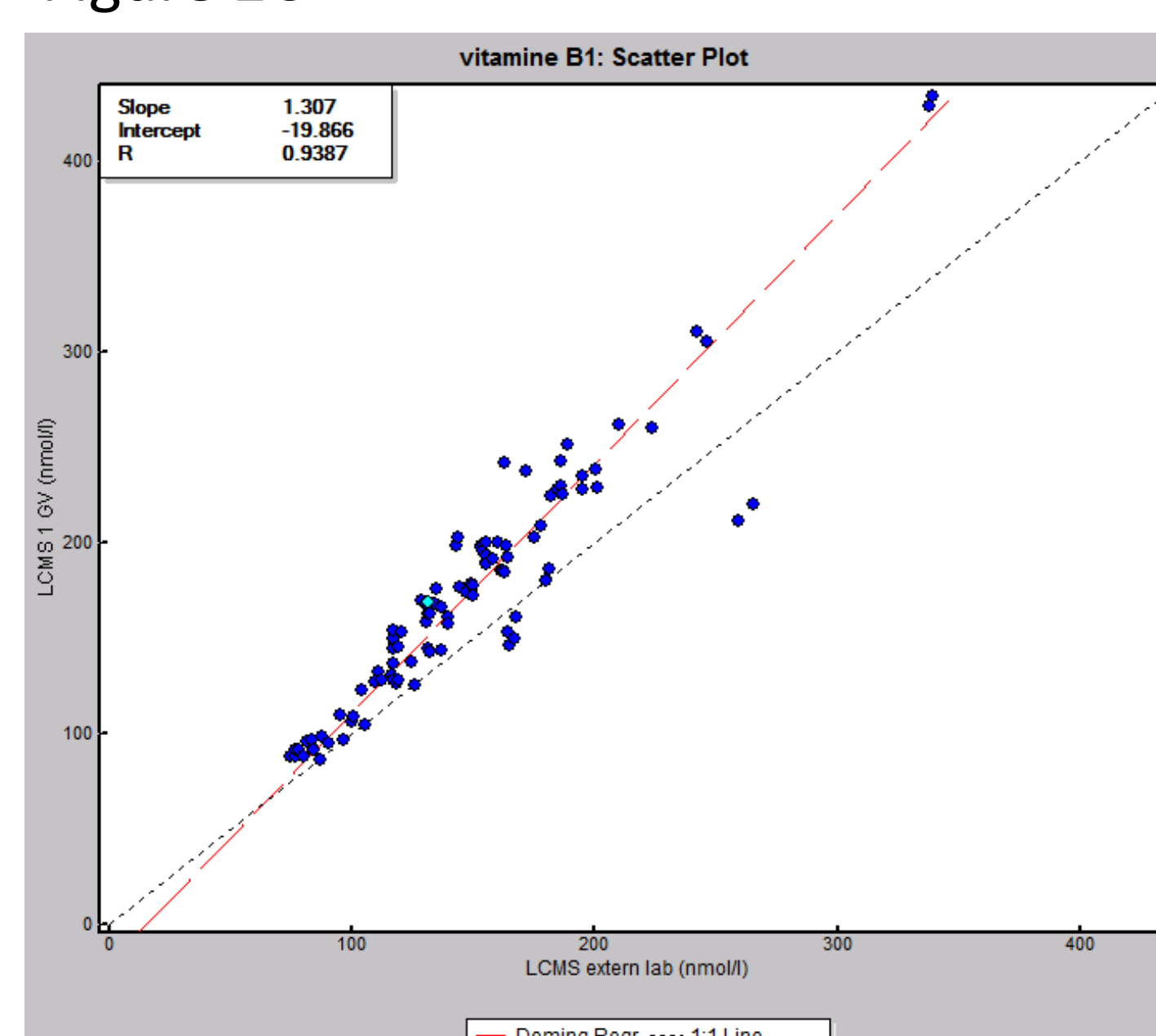


Figure 2D

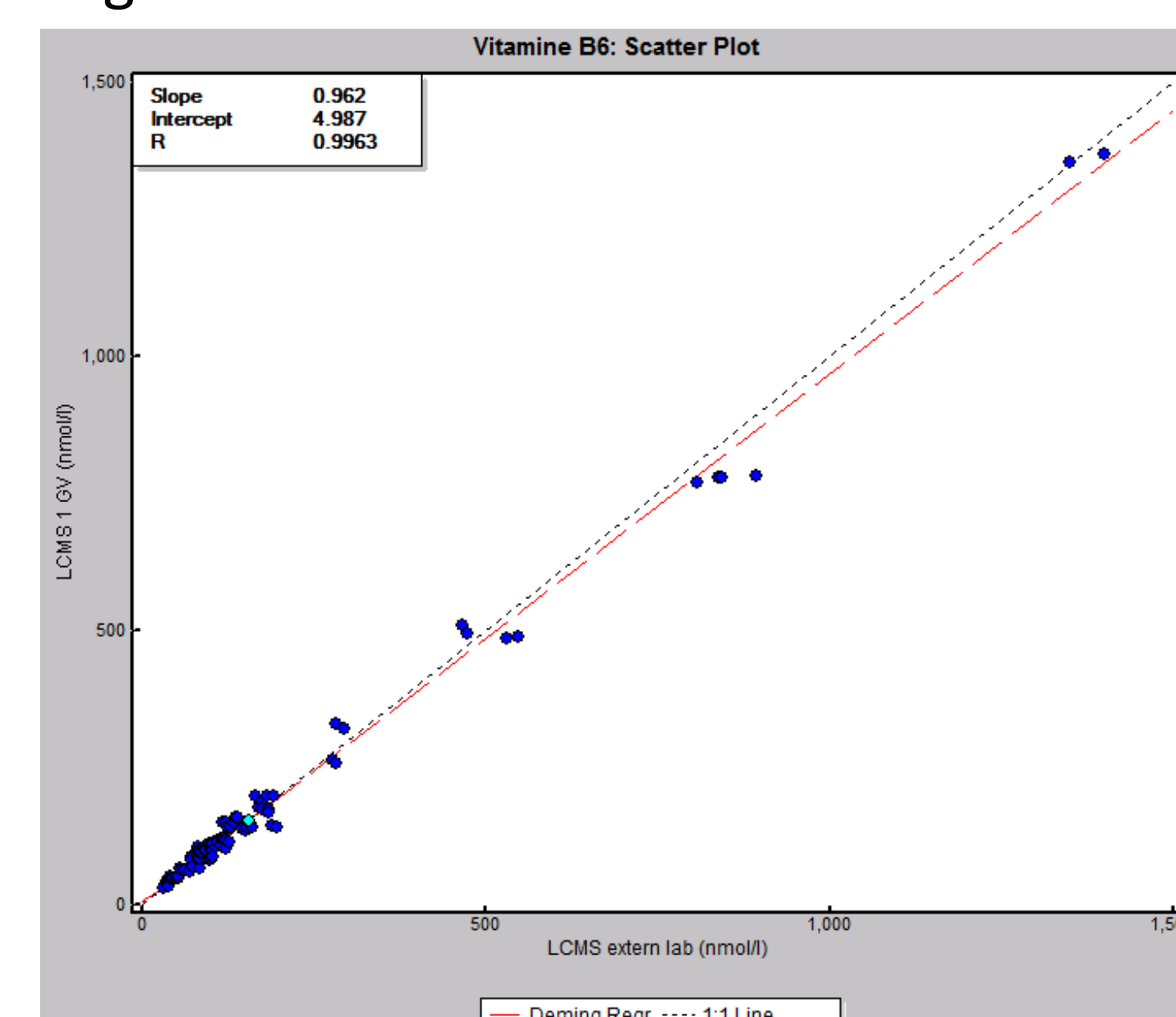


Figure 2: Deming regression scatterplots. Method comparison was performed between HPLC-LCMS for vitamin B1 (2A) and B6 (2B), and between LCMS-external lab LCMS for vitamin B1 (2C) and B6 (2D).

