



Quantitative Determination of Human IgA Subclasses and their Fc-Glycosylation Patterns in Human Plasma by Using Peptide Analogue Internal Standard and UHPLC-MS/MS

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Introduction

Glycosylation of human immunoglobulins plays an important role in immunity. Abnormal glycosylation on endogenous proteins may cause autoimmune diseases. Immunoglobulin A(IgA) nephropathy is one of the most-known autoimmune diseases, which was found to have IgA and IgA-IgG complex deposition in the glomerular mesangial cells. It was also found that there is deficiency in galactose of some of the O-linked glycans at IgA1 hinge region which causes autoimmunity. No special reports of IgA N-glycosylation for IgAN patients. As a result, it is important to have an analytical workflow to monitor IgA related glycosylation patterns for clinical application. In this study, we aim to to purify human IgA by adequate IgA binder to achieve accurate quantification of IgA glycosylation in an UHPLC-MS/MS system. In the current progress, multiple reaction monitoring mode was developed for monitoring N-glycosylation peptides from human IgA.

Results

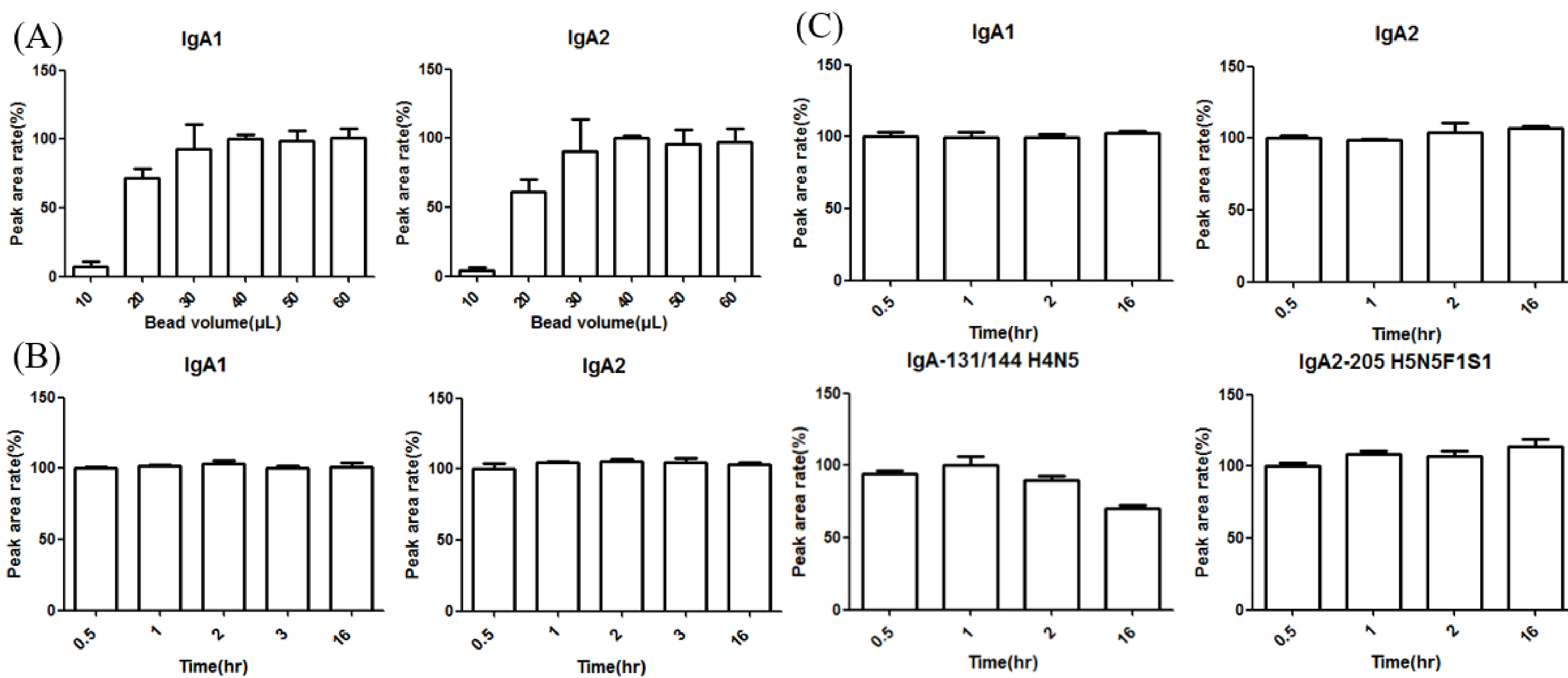


Figure 1. Optimization of IgA purification and on-bead digestion.

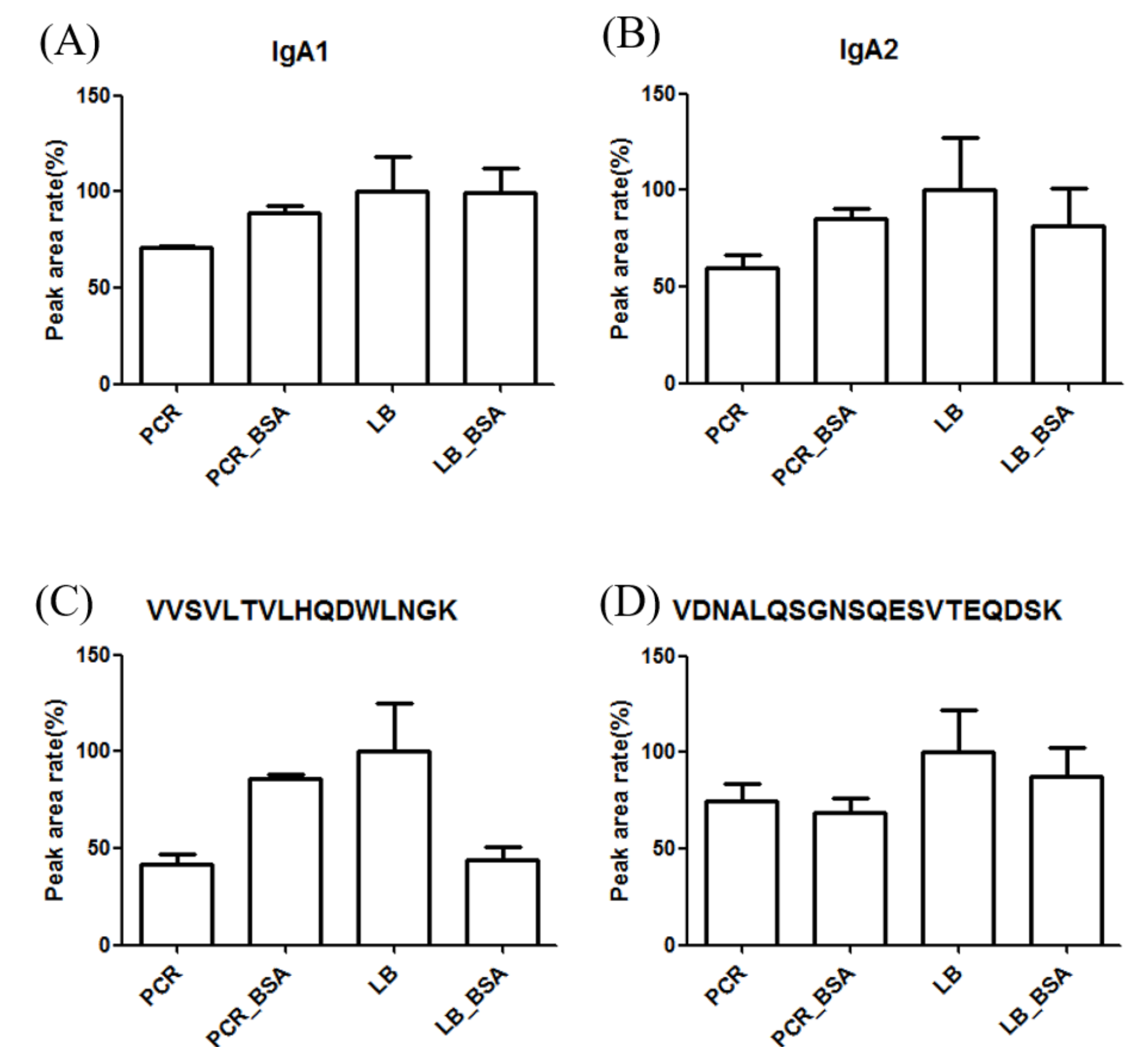


Figure 2. Attenuate non-specific protein and peptide binding.

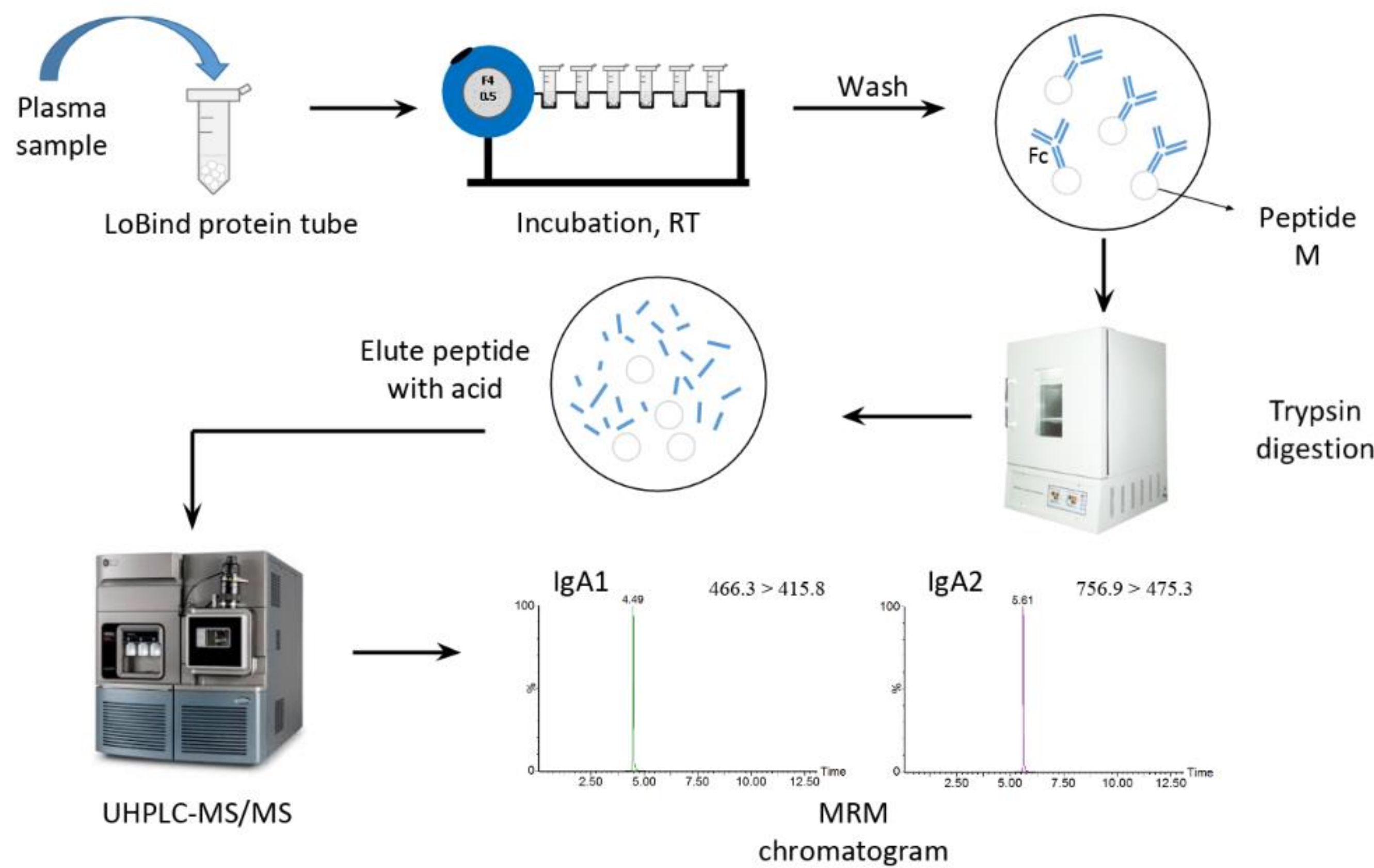


Figure 3. Workflow of IgA purification and UHPLC-MS/MS analysis.

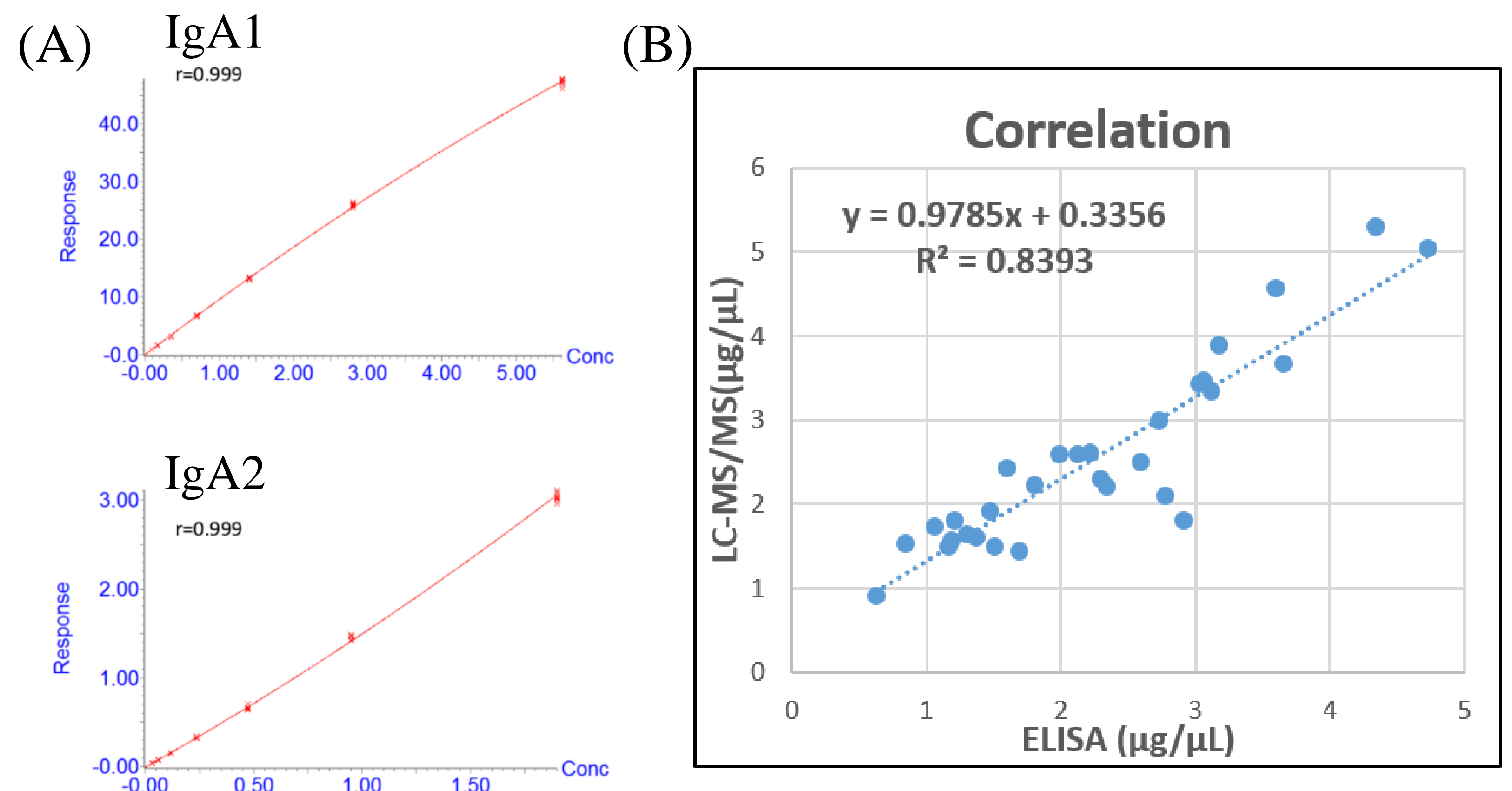


Figure 4. (A) Calibration curve for IgA1 and IgA2. (B) Comparison of results from ELISA and LC-MS/MS for 30 clinical samples.

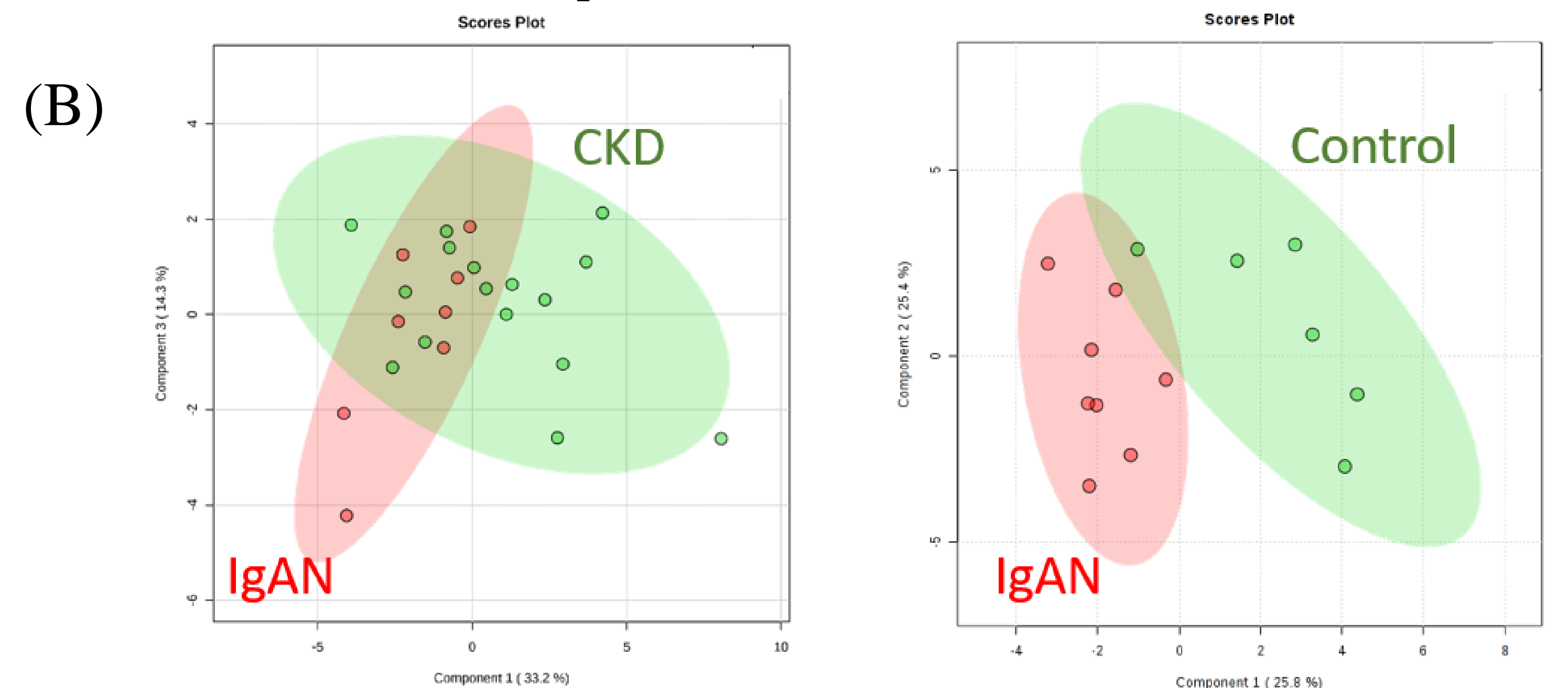
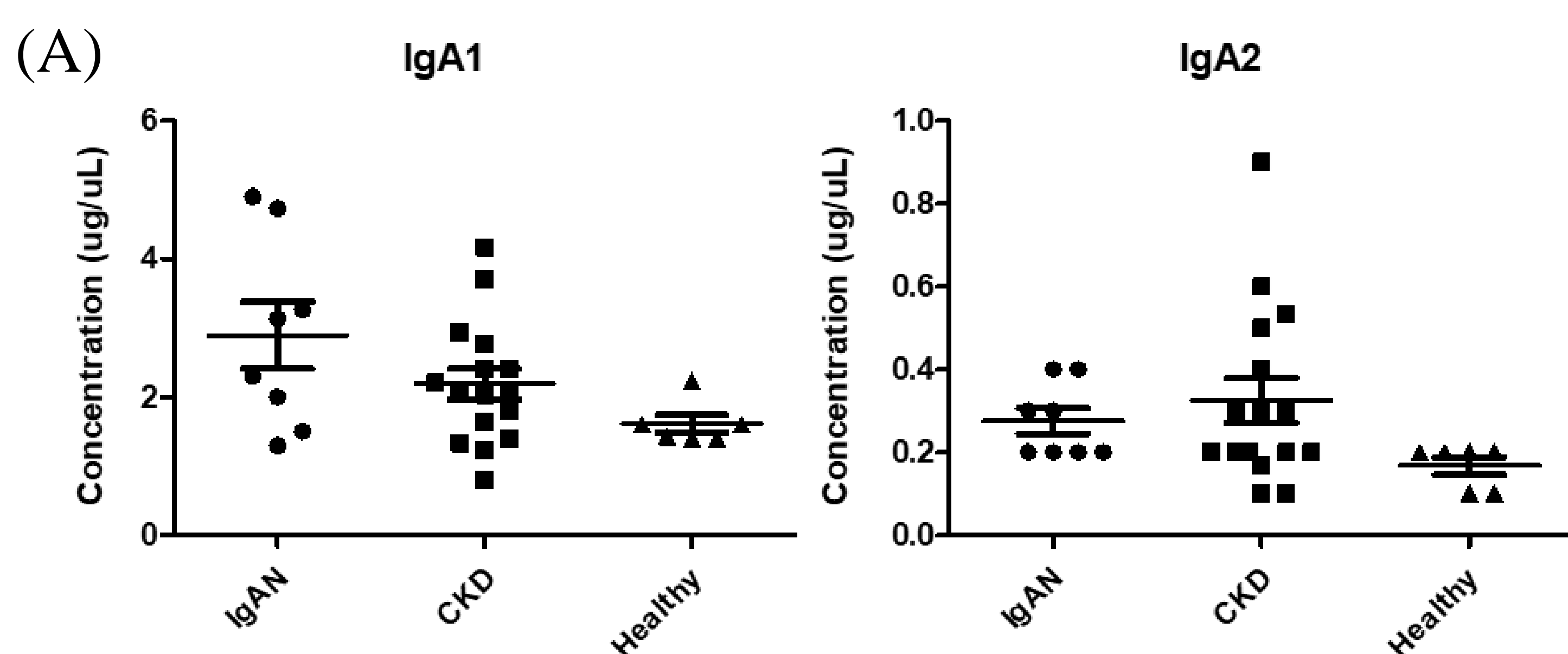


Figure 5. Clinical applications. (A) Distribution of IgA1 and IgA2 concentrations among three groups. (B) Partial least squares-discriminant analysis (PLS-DA)-IgAN v.s. CKD groups (left panel) and IgAN v.s. control groups (right panel).

Conclusion

In this study, we have developed IgA1 and IgA2 quantification method coupled with peptide M purification and an efficient on-bead protein digestion using UHPLC-MS/MS analysis. This method can be applied to more clinical samples with IgA-related diseases.