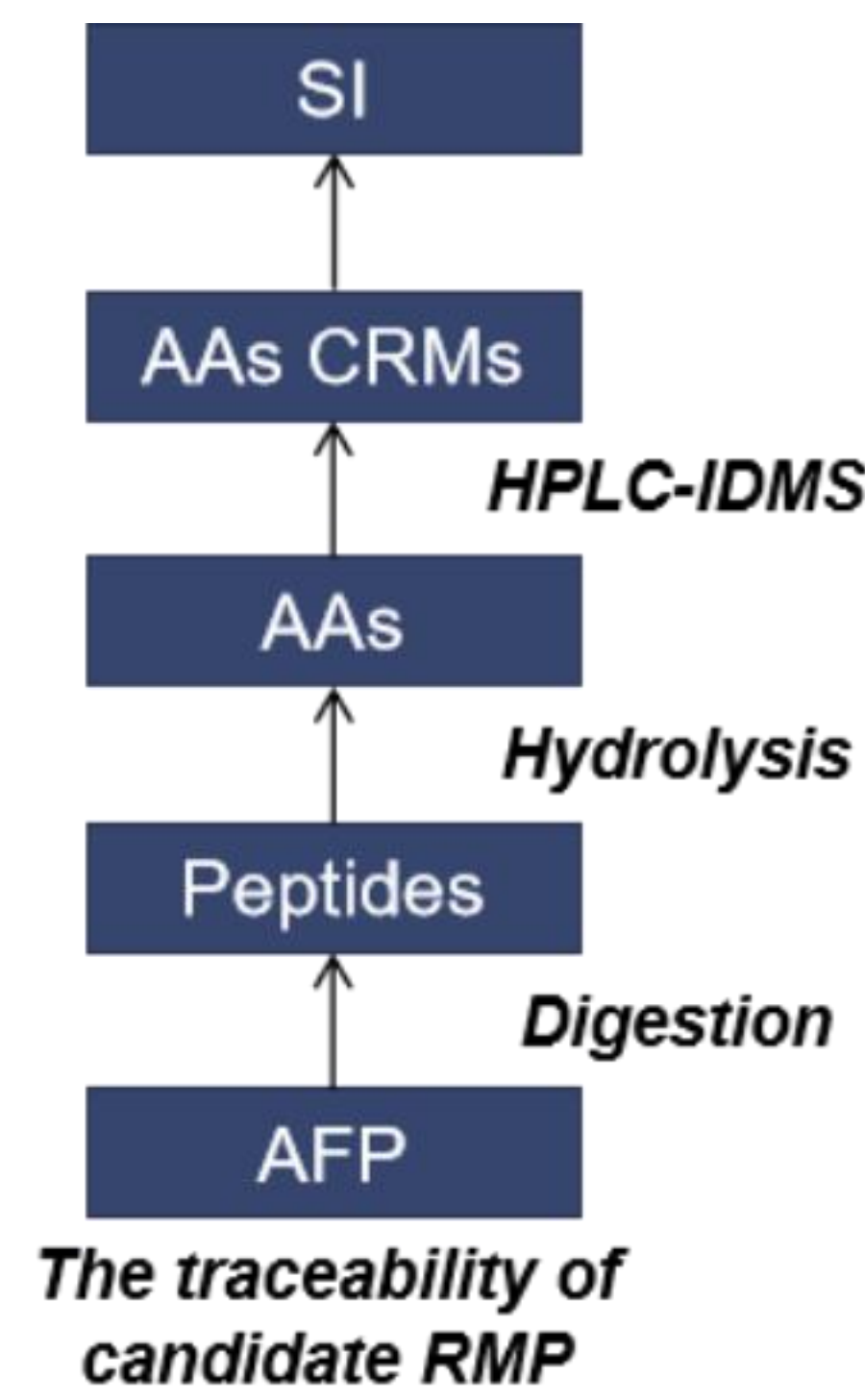


Candidate Reference Procedure for the Quantification of Alpha-Fetoprotein in Serum Based on Isotope Dilution Mass Spectrometry

Introduction

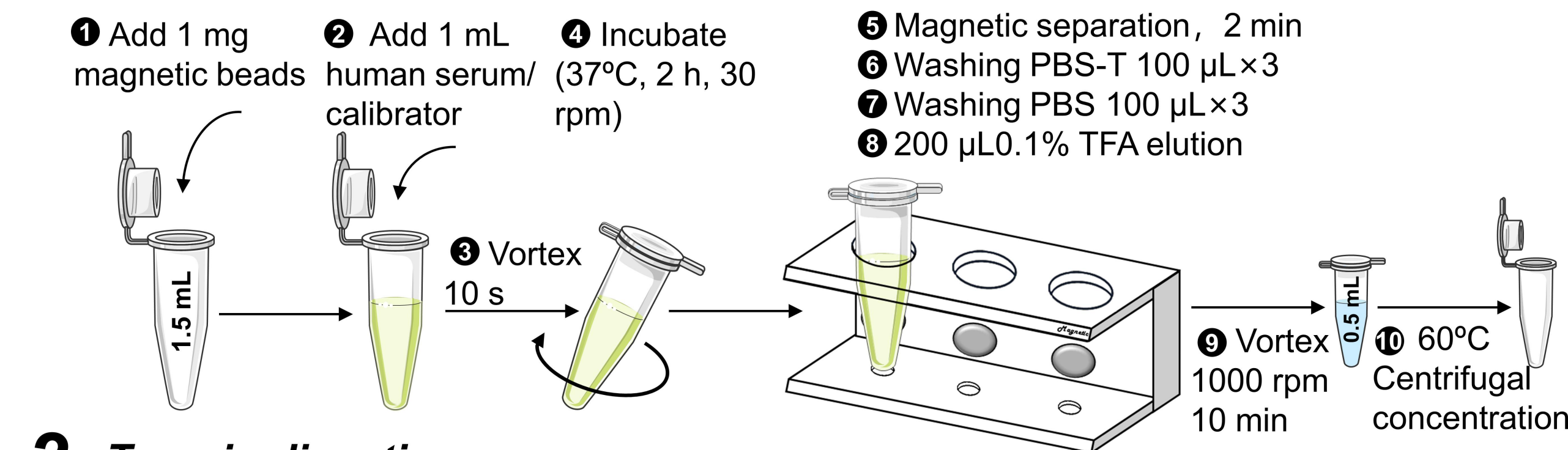
Alpha-Fetoprotein (AFP) is a glycoprotein belonging to the albumin family, primarily synthesized by the yolk sac, liver, and gastrointestinal tract during embryonic development. Its molecular weight is approximately 70 kDa. In general, the serum AFP level in healthy adults is typically below 10 ng/mL, while in liver cancer patients, it can increase to 200 ng/mL. AFP is one of the earliest discovered tumor markers and is also used for monitoring pregnancy, chronic liver diseases, and other conditions.

Therefore, the clinical measurement of AFP is of great importance. Clinical detection of AFP is typically performed using immunoassay methods; however, in practice, these methods often lack consistency across different platforms. Hence, there is an urgent need to develop a highly accurate quantitative method for serum AFP based on mass spectrometry.



Methods

1 Immunoaffinity purification



2 Trypsin digestion

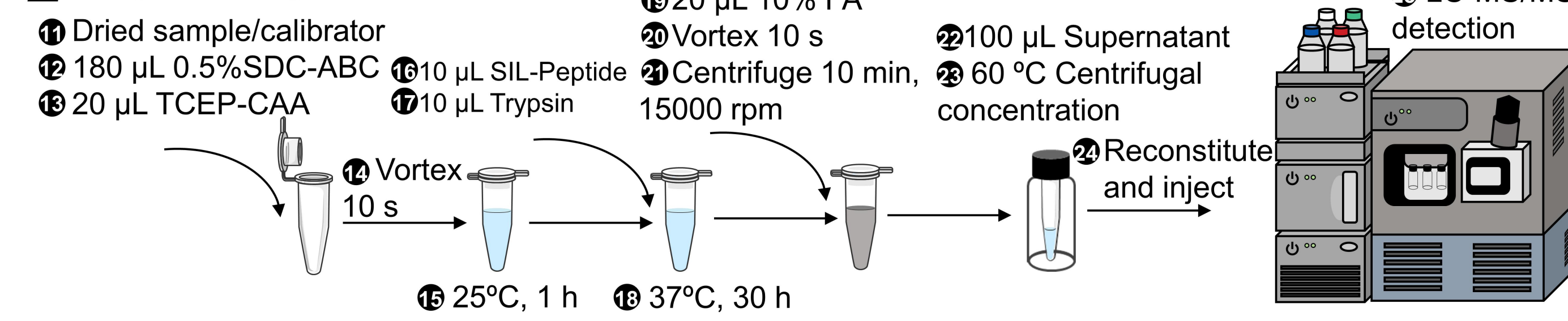


Figure 1. Candidate RMP for Serum AFP Quantification by MSPE-HPLC-IDMS

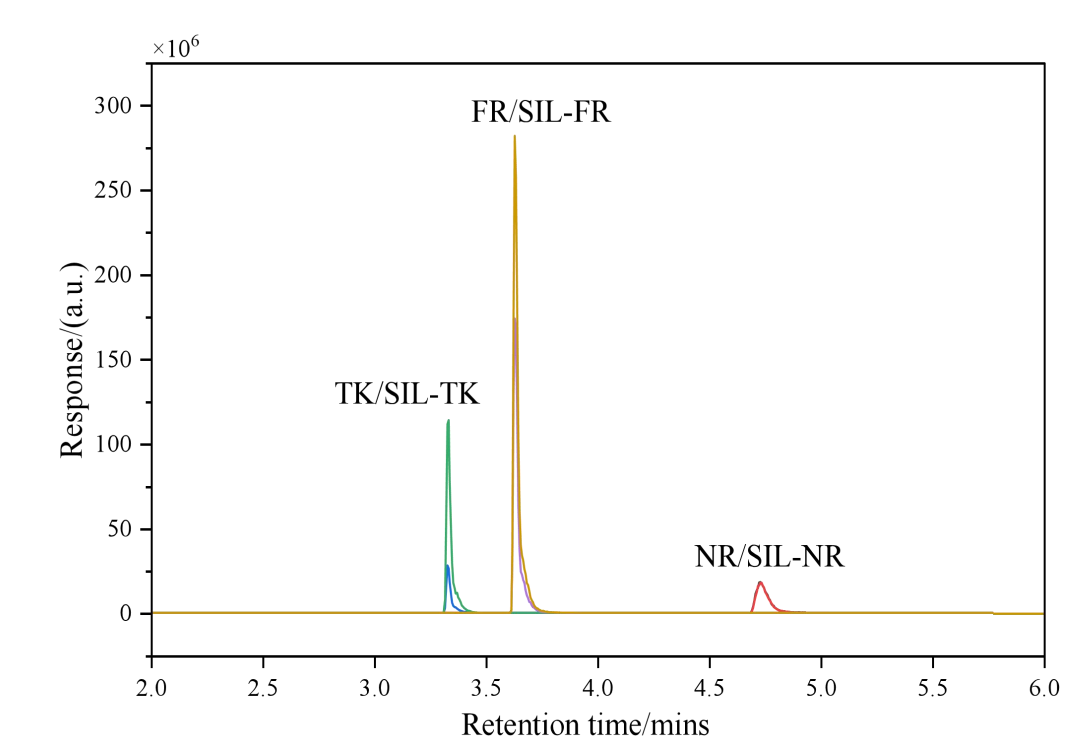


Figure 2. The extracted ion chromatogram (EIC) of sample

LC Conditions

Column: ACQUITY HSS T3 (2.1×100 mm, 1.8 µm)
Gradient: 0.1% formic acid in water/acetonitrile over 15 min.

MS Conditions

Ionization: ESI+
Detection: MRM transitions FR 456.5→651.4, SIL-FR 461.5→661.4, TK 505.1→760.6, SIL-TK 509.0→768.6, NR 528.7→678.7, and SIL-NR 532.0→683.6

Optimization of the MSPE method

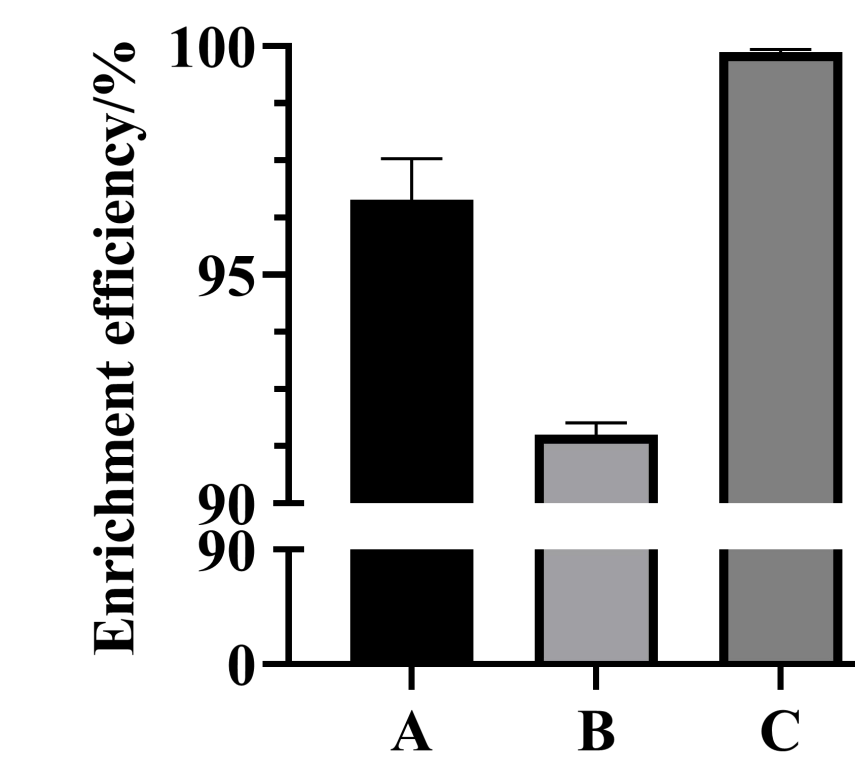


Figure 3. The effect of MSPE extractant on enrichment efficiency

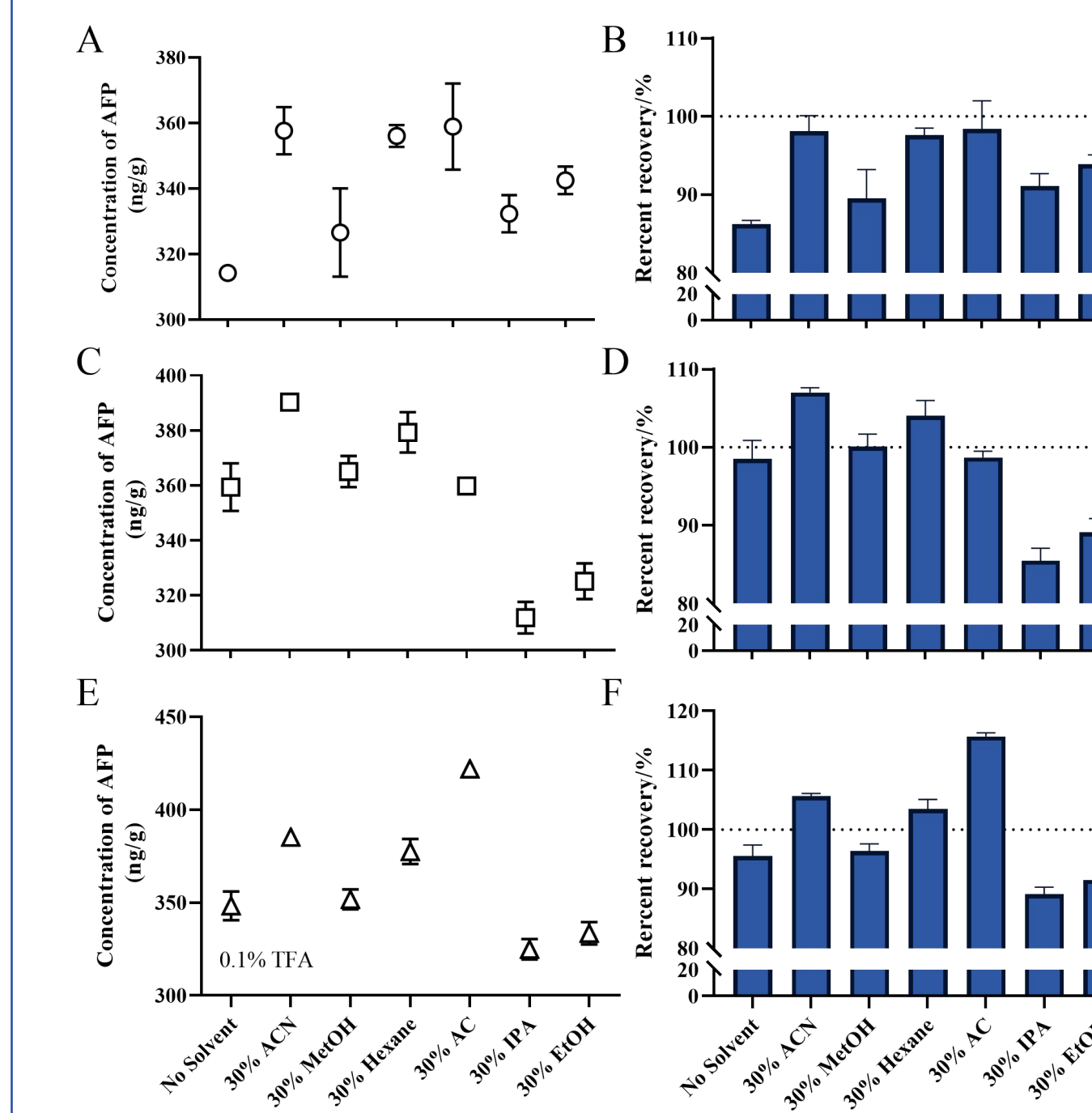


Figure 4. The effect of elution solvent composition on elution efficiency.

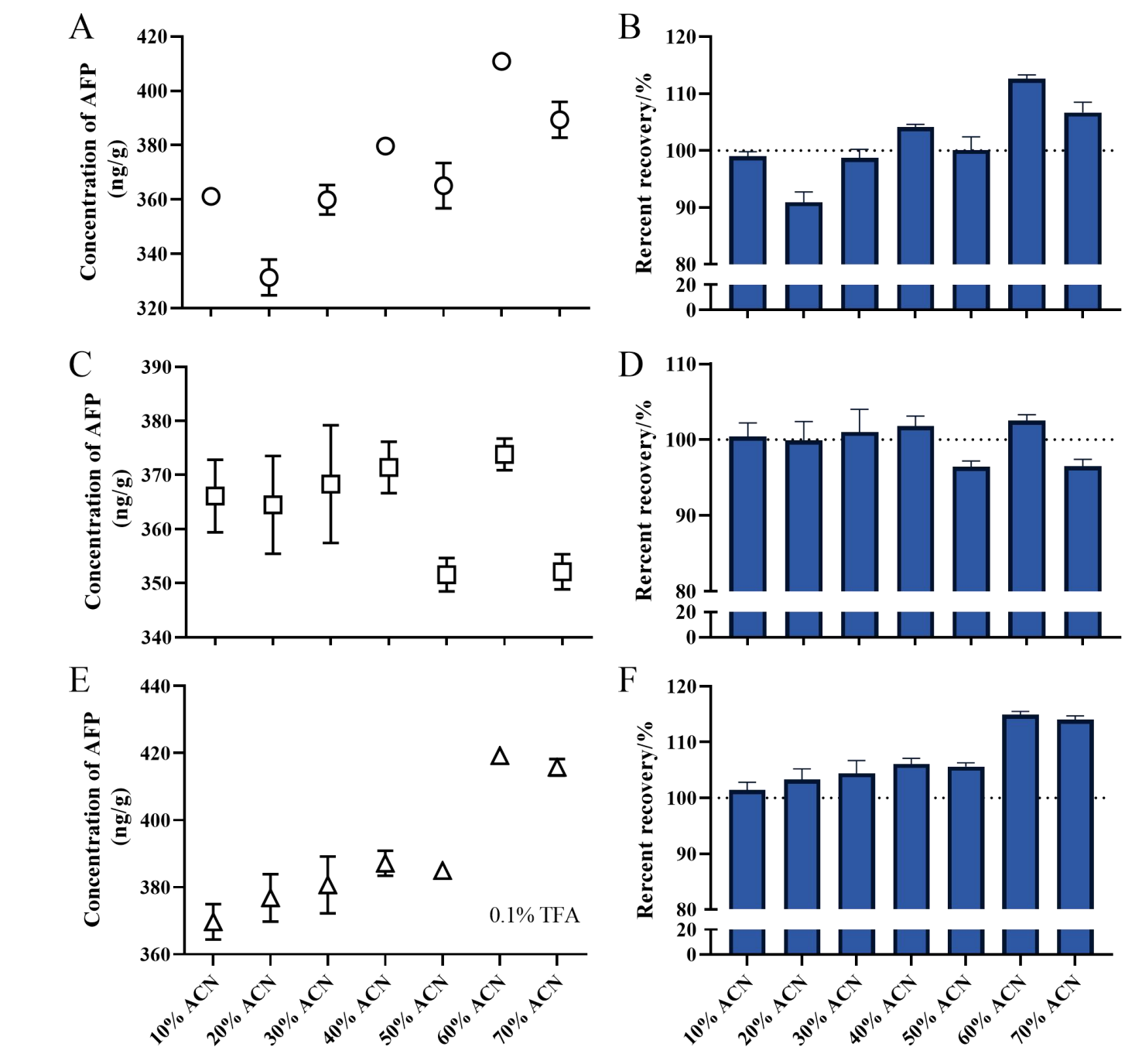


Figure 5. The effect of the organic phase addition ratio on elution efficiency

Optimization of enzymatic digestion time

Test the AFP concentration of 40 ng/mL in pregnant women's serum samples using the established method, and conduct a study on the enzymatic digestion time course to determine the shortest time required to achieve maximum enzymatic digestion efficiency. The time points set are 6, 12, 24, 36, and 48 hours.

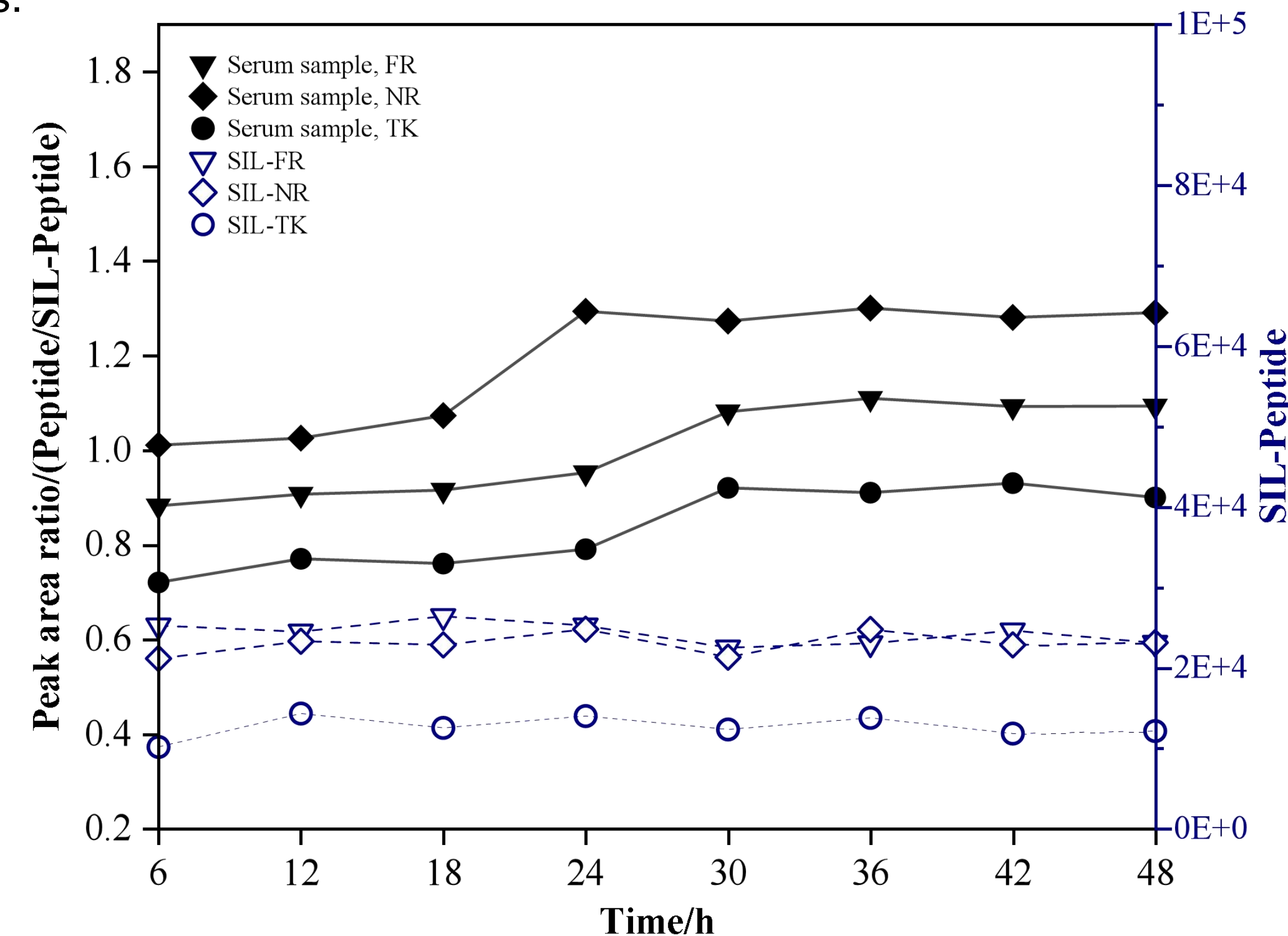


Figure 6. Kinetics of serum AFP enzymatic digestion

Method performance validation

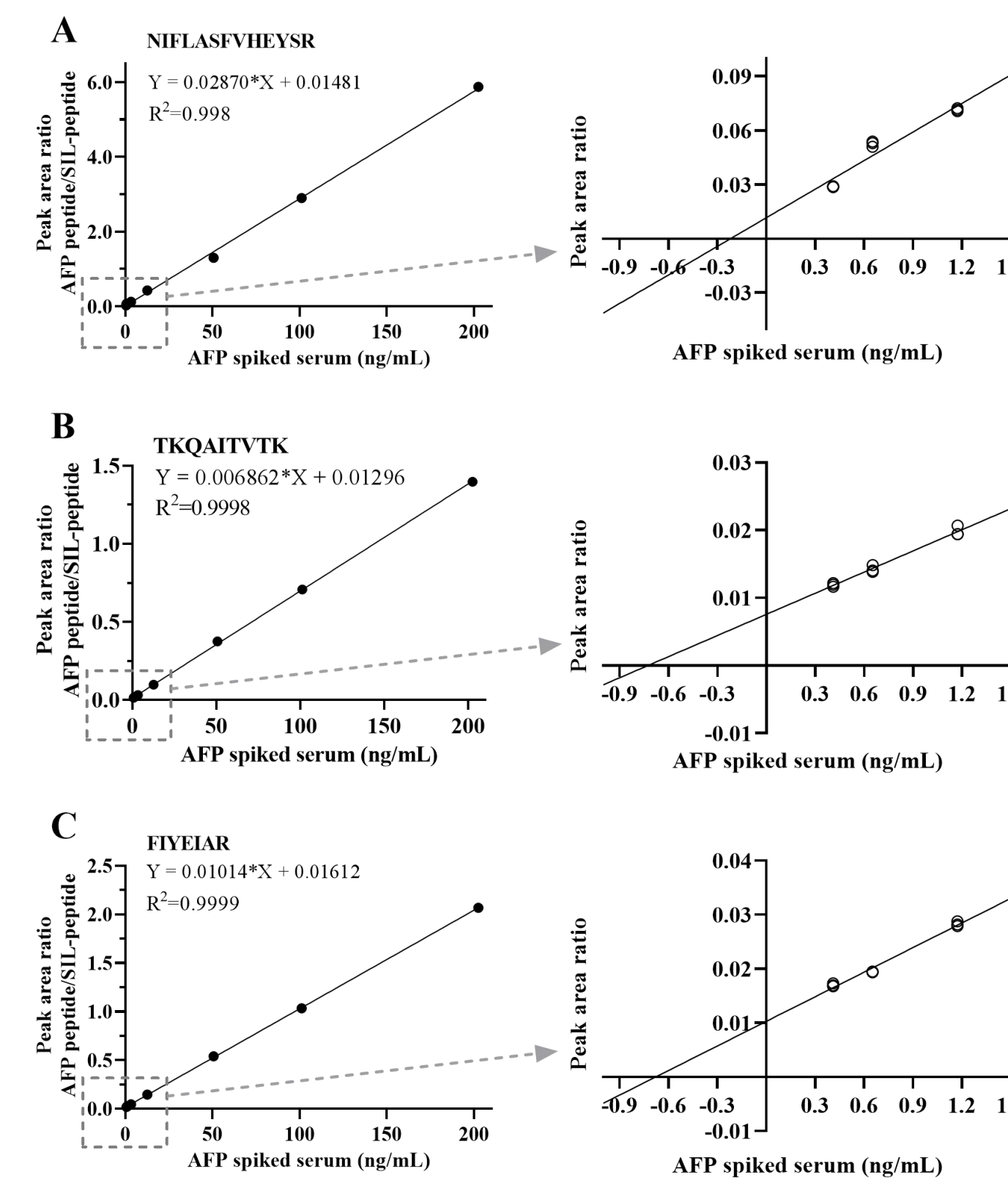


Figure 7. Linearity of the candidate RMP

Table 1. Spiked recovery test of the sample

Spiked AFP/(ng/mL)	Measured AFP/(ng/mL)	Recovery rate/%	RSD/%
364.8	365.7	99.8	1.3
89.6	90.4	100.9	2.9
43.6	44.2	101.4	3.8

Using 5 wt% BSA as the substitute matrix, prepare seven concentrations of AFP serum pools by diluting the AFP solution reference materials (GBW09227). Measure the peak area ratios of the samples within the concentration range of 0.4-200 ng/g. The linear fitting equation between concentration and measured signal is obtained through regression analysis to evaluate the method's linearity.

Conclusion

A quantitative method for AFP in serum based on MSPE-HPLC-IDMS was established. This method utilizes high-affinity AFP antibodies and magnetic nanoparticles for enrichment, significantly improving the recovery rate of low-abundance AFP, and ensures accuracy by optimizing the elution solution. Combined with HPLC-IDMS technology, AFP quantification was achieved with high precision through three peptide segments, and the results are traceable to the International System of Units (SI). Performance validation shows that the method has high accuracy and can serve as a candidate reference method for AFP measurement.

It provides new insights for the development of serum AFP reference materials and offers an effective technical approach for the quantification of low-abundance proteins in serum.

References

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