

Feasibility of LC–MS/MS Based Therapeutic Drug Monitoring for Vedolizumab and Ustekinumab

Using Commercial PK and ADA Kits



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INTRODUCTION

- Vedolizumab and Ustekinumab are widely used monoclonal antibodies (mAbs) for the treatment of inflammatory bowel disease, and therapeutic drug monitoring (TDM) supports clinical management.
- Quantitation from immunoassay-based drug and anti-drug antibody testing (ADA) can be limited by analytical interference and inter-assay variability.
- Liquid chromatography–tandem mass spectrometry (LC–MS/MS) measures drug-specific peptides, improving analytical specificity.
- We assessed commercial LC–MS/MS pharmacokinetic (PK) and ADA kits for clinical laboratory use.
- Kits (mAbXmise®) are developed by Promise Proteomics (France) and manufactured under ISO13485, recognized by the FDA (since Feb 2026).
- Patents: WO2023156583, WO2017077081, WO2017077080.

METHODS

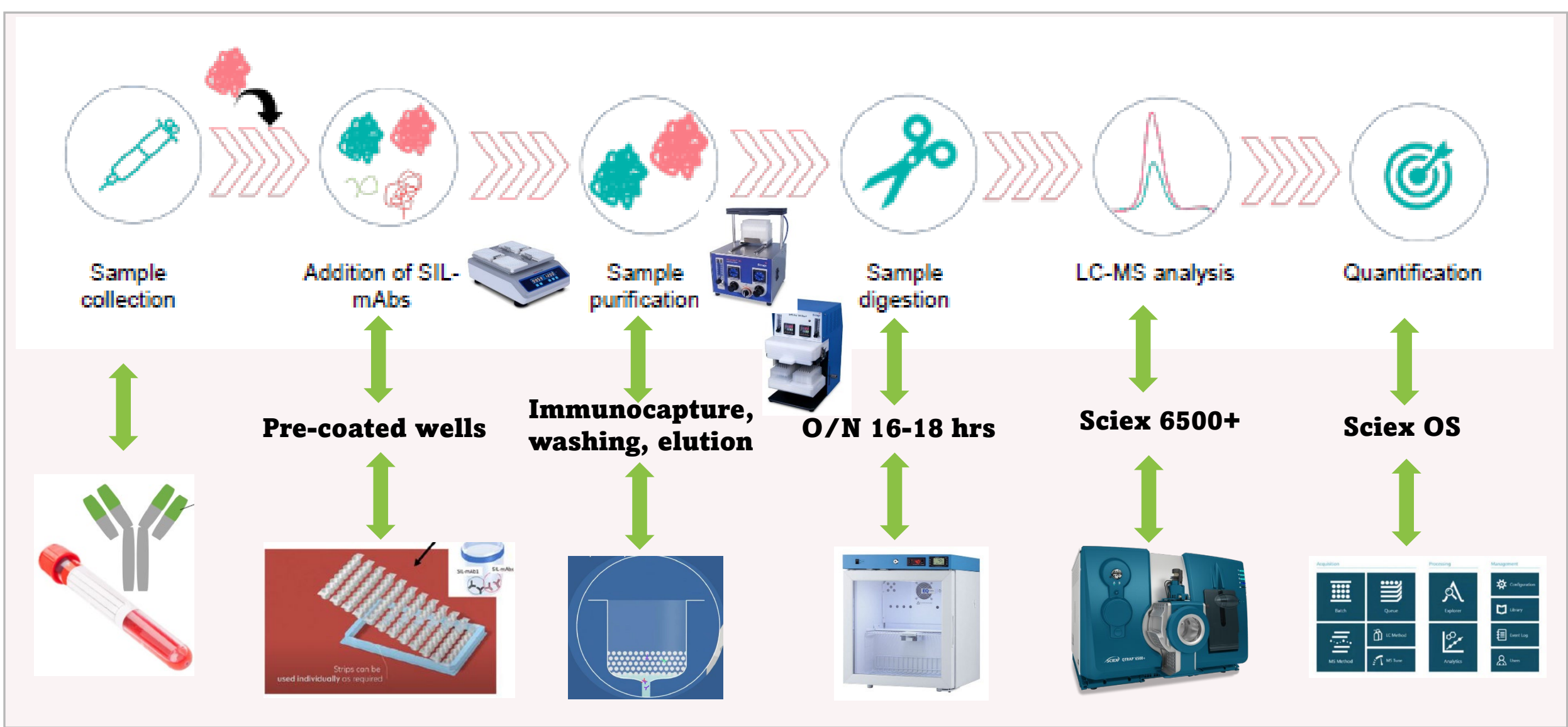


Figure 1. Analytical workflow for the extraction of Vedolizumab and Ustekinumab PK using the mAbXmise ITDM2 kit.

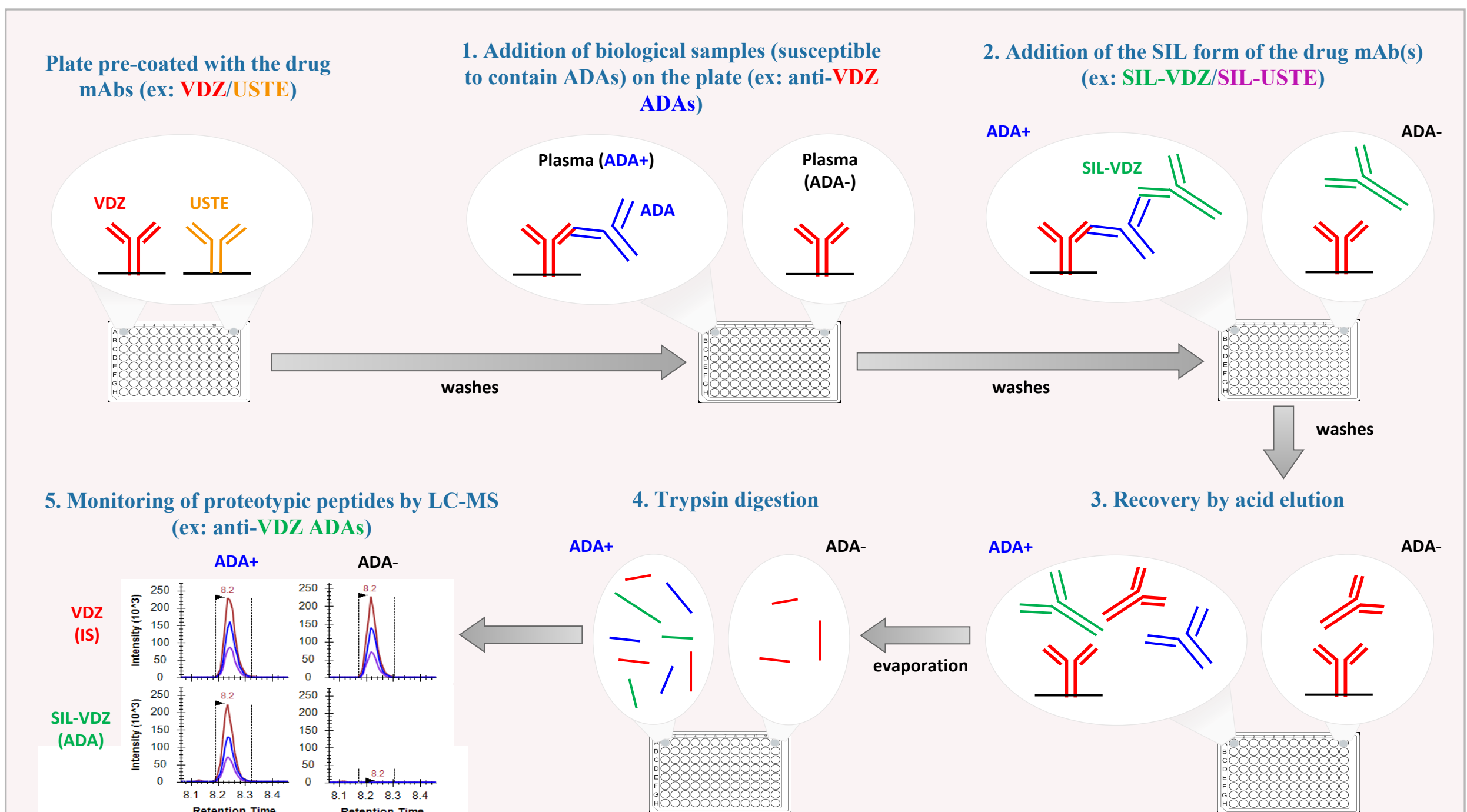


Figure 2. Analytical workflow for the extraction of Vedolizumab and Ustekinumab ADA using the mAbXmise IADA2 kit.

LC-MS/MS Method Details

- ❖ Agilent 1290 Infinity II with SCIEX 6500+ QQQ
 - ❖ Injection volume: 10 µL (PK), 20 µL (ADA)
 - ❖ MPA: 0.1% formic acid in water
 - ❖ MP B: 0.1% formic acid in acetonitrile
 - ❖ Flow rate: 300 µL/min (PK), 100 µL/min (ADA)
- (A)

(B)

Time (Min)	A (%)	B (%)
0.00	95.0	5.0
1.00	95.0	5.0
1.10	85.0	15.0
2.50	70.0	30.0
4.50	50.0	50.0
4.60	10.0	90.0
6.00	10.0	90.0
6.10	95.0	5.0
7.50	95.0	5.0

Time (Min)	A (%)	B (%)
0.00	95.0	5.0
1.00	95.0	5.0
5.50	60.0	40.0
5.60	10.0	90.0
6.70	10.0	90.0
6.80	95.0	5.0
8.00	95.0	5.0

Table 1. Mobile phase gradient used in the method for eluting pump (A) PK (B) ADA.

RESULTS

Vedolizumab and Ustekinumab PK performance was evaluated for reference laboratory comparison; Vedolizumab showed good agreement with an LC–MS/MS method across 84 de-identified patient samples, with a Deming regression slope of 1.40 (95% CI 1.34–1.48) and R of 0.976; Bland–Altman analysis showed a mean positive bias of 10 µg/mL (46%, Figure 3). Ustekinumab was compared with a reference laboratory immunoassay method across 44 de-identified patient samples, yielding a Deming regression slope of 1.13 (95% CI, 0.96–1.30) and an R of 0.899; Bland–Altman analysis showed a mean positive bias of 0.28 µg/mL (5%, Figure 4).

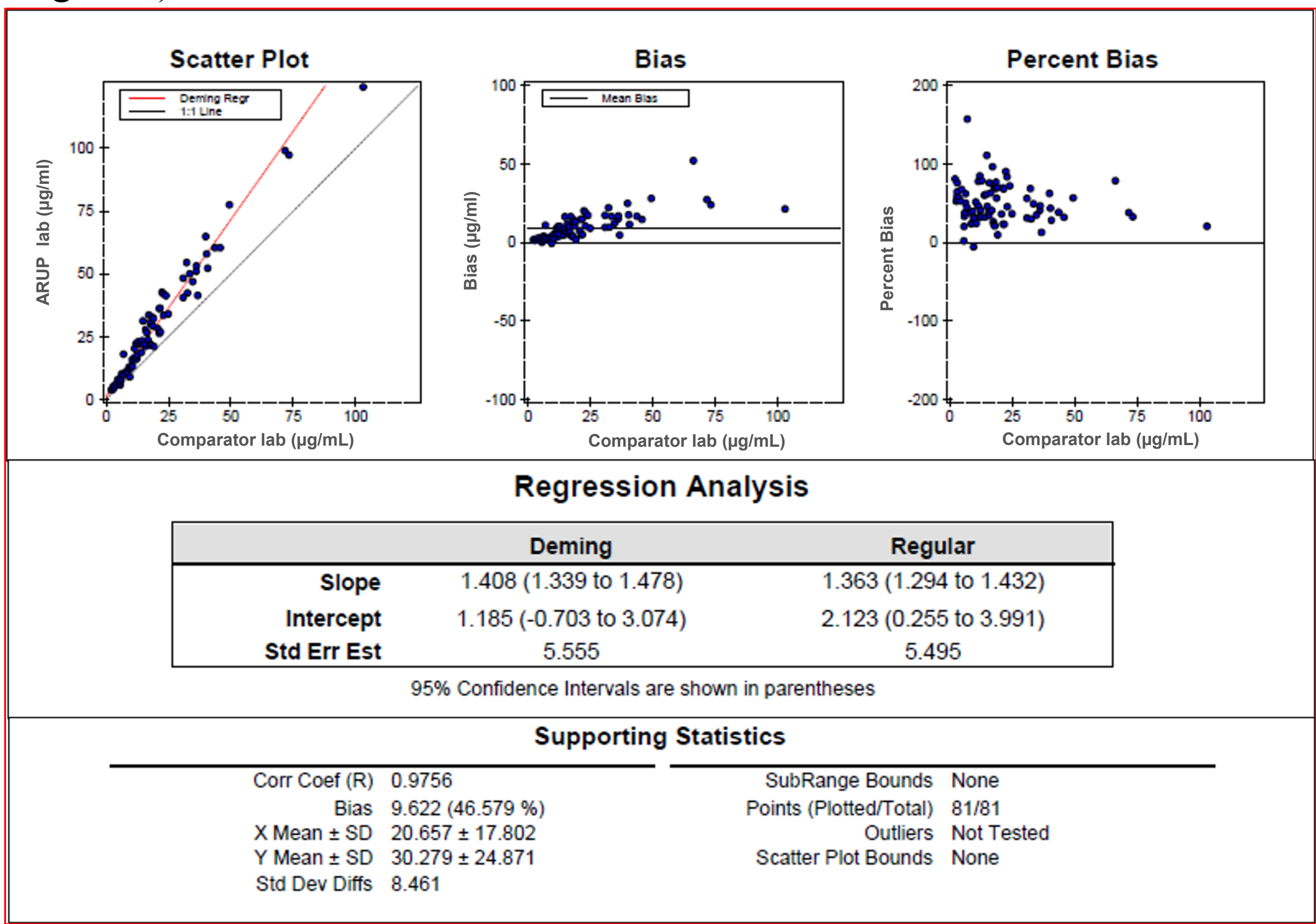


Figure 3. Method comparison (Accuracy): Deming Regression for Vedolizumab PK.

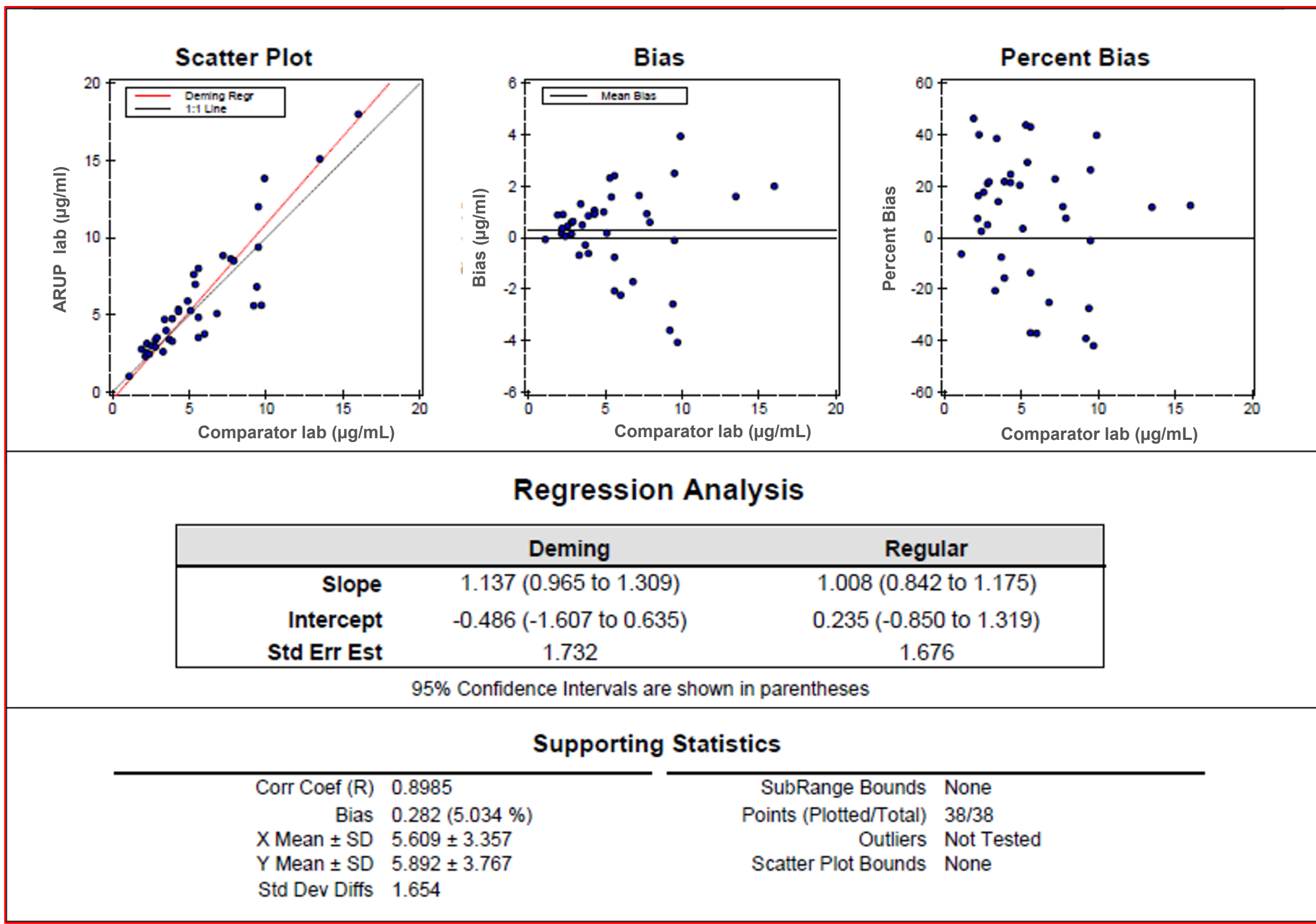


Figure 4. Method comparison (Accuracy): Deming Regression for Ustekinumab PK.

Linearity was demonstrated for Vedolizumab across 2.3–88 µg/mL (Figure 5A) using spike-in and dilution studies and for Ustekinumab from 2.5–101 µg/mL (Figure 5B).

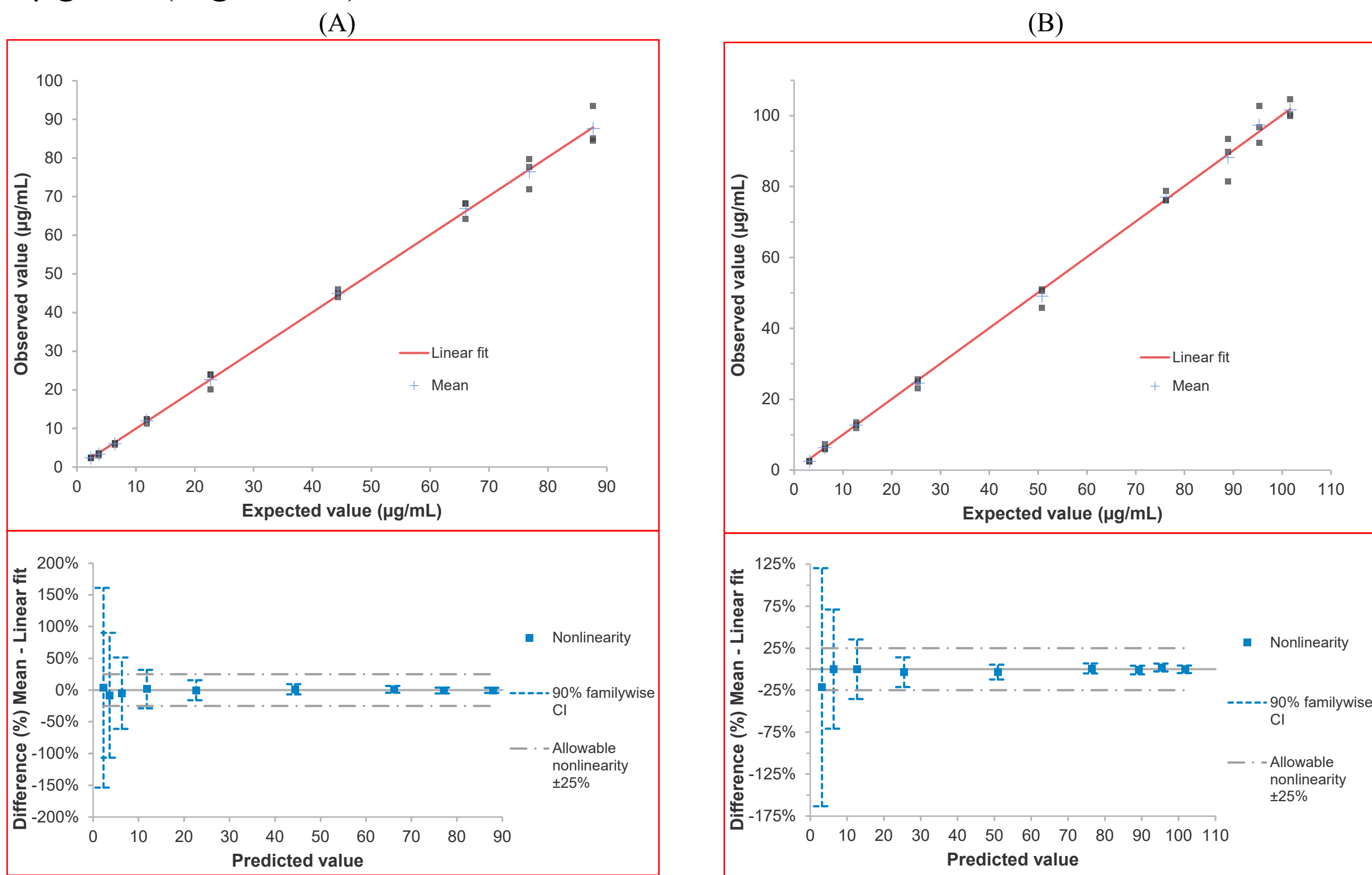


Figure 5A-B. Evaluation of the method linearity (A) Vedolizumab PK (B) Ustekinumab PK using mAbXmise kits and LC-MS/MS.

Precision testing showed acceptable performance for both analytes (Table 2). Vedolizumab PK total CVs were 3% at low concentrations (4–6 µg/mL) and <4% at mid-to-high concentrations (30–50 µg/mL), while Ustekinumab PK total CVs were 10% at low concentrations (3–4 µg/mL), 4% at mid concentrations (7–9 µg/mL), and 13% at higher concentrations (15–18 µg/mL).

Vedolizumab PK	Within-run % CV	Between-run/Day % CV	Total % CV	Average µg/mL	Expected µg/mL	% Accuracy
Low	2.70	1.50	3.09	5.98	5.91	101%
Mid	0.75	0.35	0.82	30.2	30.3	100%
High	3.86	0.25	3.87	48.3	48.4	100%

Ustekinumab PK	Within-run % CV	Between-run/Day % CV	Total % CV	Average µg/mL	Expected µg/mL	% Accuracy
Low	10.3	1.53	10.50	3.40	3.36	101%
Mid	2.68	3.55	4.45	7.90	7.70	103%
High	8.85	9.77	13.2	15.1	14.0	107%

Table 2. Summary on method imprecision and accuracy for Vedolizumab and Ustekinumab PK.

Table 3 summarizes the results of the Vedolizumab ADA comparison using an external laboratory method and an internal ELISA kit. The mAbXmise IADA2 kit easily detected the two patients with high Vedolizumab ADA concentrations, and the patient with the lowest Vedolizumab ADA concentration (10.3 ng/mL) was below the LOQ. However, it is concerning that the patient with the modest ADA concentration of 178 ng/mL had no detectable ADA by the mAbXmise IADA2 kit, although it is possible that the free vedolizumab present (7.6 µg/mL) may be impeding the detection of ADA.

Vedolizumab ADA	Comparator Lab ADA Conc. (ng/mL)	IDK ELISA ADA Conc. (AU/mL)	LC-MS/MS ADA (ng/mL)
De-identified_Pt_Sp_01	>1250	558.4	1091.1
De-identified_Pt_Sp_02	10.3	<10	6.58
De-identified_Pt_Sp_03	178	<10	<10
De-identified_Pt_Sp_04	>1250	567	984.2

Table 3. Summary of the results of the method comparison for Vedolizumab ADA.

Vedolizumab LC–MS/MS ADA assays detected high-titer antibodies and were linear over the range of 11–500 ng/mL, with average calibrator recoveries near 100%. Ustekinumab ADA demonstrated linearity over the range of 20–470 ng/mL.

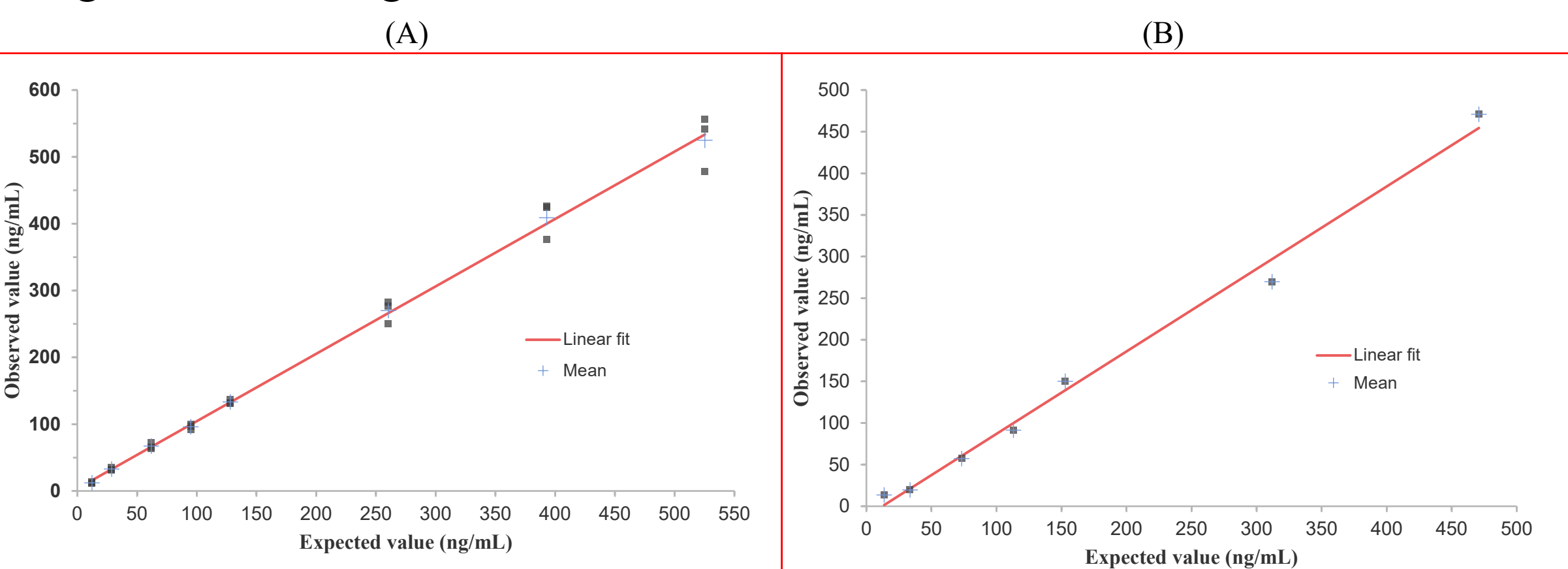


Figure 6 A-B. Evaluation of the method linearity (A) Vedolizumab ADA (B) Ustekinumab ADA using mAbXmise IADA2 kit and LC-MS/MS.

Precision across ADA concentration pools ranged from 2–10% CV at low to mid-levels and 8–16% CV at higher concentrations for both analytes.

Vedolizumab ADA	Peptide conc. (ng/mL)	Avg. Conc. (ng/mL)	STDEV S	CV%	Ustekinumab ADA	Peptide conc. (ng/mL)	Avg. Conc. (ng/mL)	STDEV S	CV%
Low_1	114.0	102.0	10.0	9.84%	Low_1	54.4	51.8	5.3	10.3%
Low_2	89.6				Low_2	44.3			
Low_3	102.0				Low_3	56.6			
Mid_1	364.0	356.0	6.4	1.80%	Mid_1	156.0	153.0	3.7	2.44%
Mid_2	348.0				Mid_2	148.0			
Mid_3	356.0				Mid_3	156.0			
High_1	628.0	572.0	55.5	9.71%	High_1	381.0	331.0	52.5	15.9%
High_2	591.0				High_2	353.0			
High_3	496.0				High_3	258.0			

Table 4. Summary on method imprecision and accuracy for Vedolizumab and Ustekinumab ADA.

CONCLUSIONS

- ❖ Commercial LC–MS/MS PK kits showed adequate performance and workflow, supporting formal validation for longitudinal TDM.
- ❖ ADA assays detected high-titer antibodies but requires optimization and broader clinical evaluation before routine use.
- ❖ LC–MS/MS offers a specific, interference-resistant alternative to immunoassays for biologic TDM.