

Addressing pH-Dependent Lactone–Carboxylate Interconversion to Resolve Quantitation Bias in the Bioanalysis of Exatecan-Based ADCs

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INTRODUCTION

Background

➤ Topoisomerase I (TOP1) inhibitor–based antibody–drug conjugates (ADCs) have emerged as a rapidly growing class of oncology therapeutics. Camptothecin-derived payloads such as DXd and Exatecan trap TOP1–DNA cleavage complexes and have shown superior efficacy compared with earlier microtubule-based ADCs in multiple solid tumor indications. Following the clinical success of trastuzumab deruxtecan (T-DXd, DS-8201), Exatecan and its derivatives are increasingly incorporated into next-generation ADC designs, including dual-payload formats and novel linker architectures.[1]

➤ In regulated PK bioanalysis, two complementary ligand-binding assays are typically used to characterize ADC exposure and biotransformation. A total antibody (TA**b**) assay measures all circulating antibody species regardless of payload status, whereas a conjugated antibody (ADC) assay selectively quantifies payload-bearing antibody and supports interpretation of drug-to-antibody ratio (DAR), linker stability, and payload deconjugation over time. Under normal circumstances, TA**b** concentrations are expected to be equal to or higher than ADC concentrations, with TA**b**/ADC ratios close to 1.0 early after dosing and gradually increasing if deconjugation, catabolism, or target-mediated clearance preferentially affects the conjugated species. This TA**b**–ADC relationship is therefore a key internal consistency check for the PK profile of ADCs, including Exatecan-based constructs.

Problems

➤ During routine PK bioanalysis of an Exatecan-based ADC from SD rat plasma, we observed an unexpected inversion of this relationship: ADC concentrations consistently exceeded TA**b**, resulting in TA**b**/ADC ratios of approximately 0.3–0.4 across multiple time points. The anomaly was reproducible and inconsistent with the ADC design, dose levels, and anticipated biotransformation pathways, suggesting the presence of an assay-related artifact rather than a true biological phenomenon.

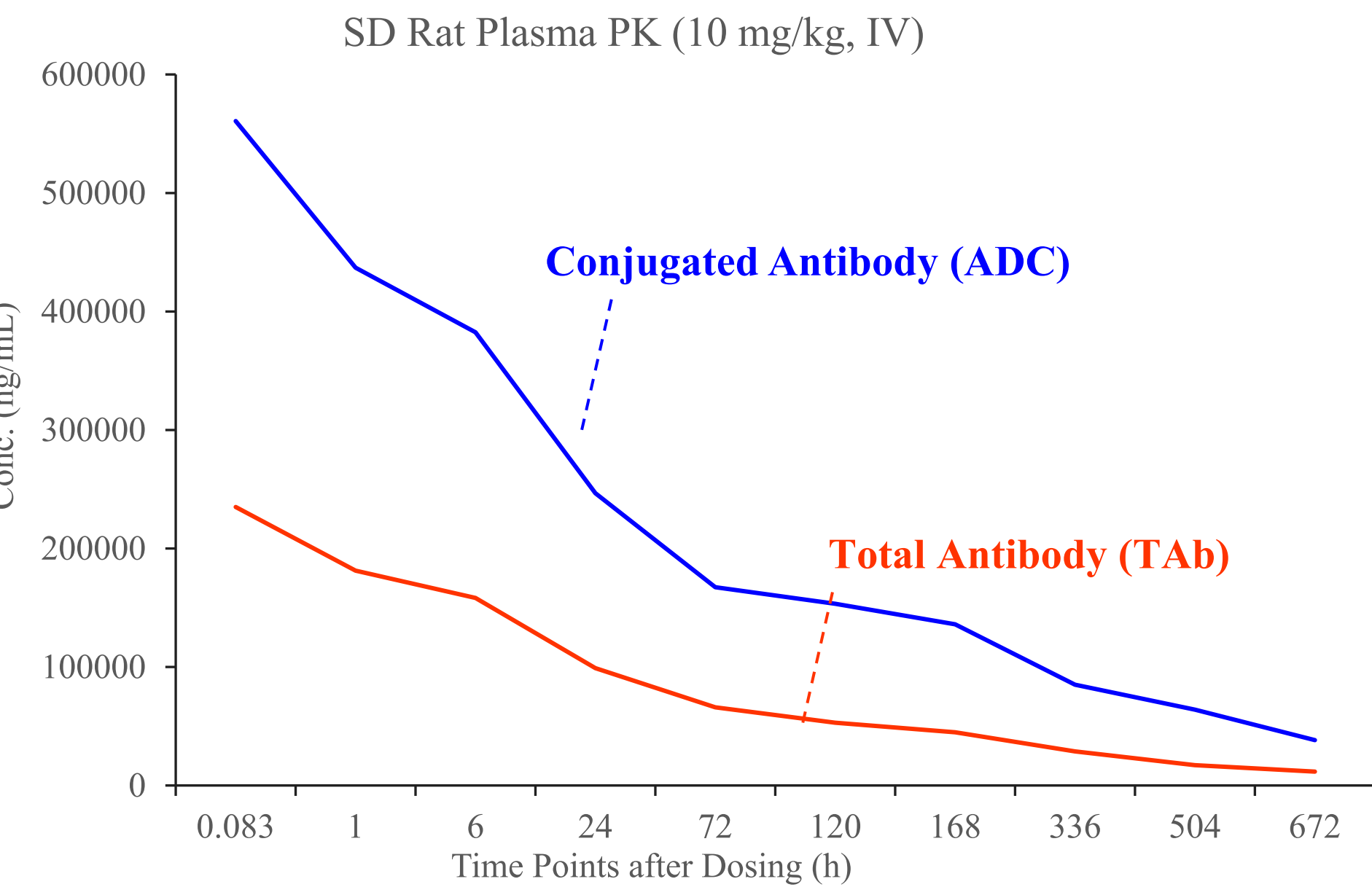


Figure 1. Total Antibody and Conjugated Antibody Concentration of an Exatecan-based ADC Trend from SD Rat Plasma

Representative Time Points	Ratio (TA b /ADC)
0.083 h #1	0.40
0.083 h #2	0.44
672 h #1	0.28
672 h #2	0.32

METHOD

Mechanistic Rationale

- Exatecan is a camptothecin analog that exists in the pH-dependent equilibrium between a closed lactone form and an open carboxylated form (Figure 2). The lactone is favored at acidic pH and the carboxylate at neutral pH, driven by reversible opening of the E-ring.
- In the conjugated form analysis through LBA, signal depends on binding of an anti-payload antibody to an epitope that includes the Exatecan moiety. Differential affinity for lactone vs carboxylated species can therefore change ADC signal even at the same true ADC concentration.
- Calibrators in buffer and PK samples in plasma/serum may have different lactone/carboxylate distributions due to pH, matrix of compounds, storage and handling, leading to mismatched speciation between standards and samples.
- Acidification is routinely used in bioanalytical workflows to stabilize labile moieties or disrupt complexes, typically with incubation times ranging from minutes to a few hours. The extent to which both the duration of acidification and the lactone–carboxylate equilibrium jointly influence ADC assay performance and ADC stability has not been systematically evaluated for Exatecan-based ADCs.[2]

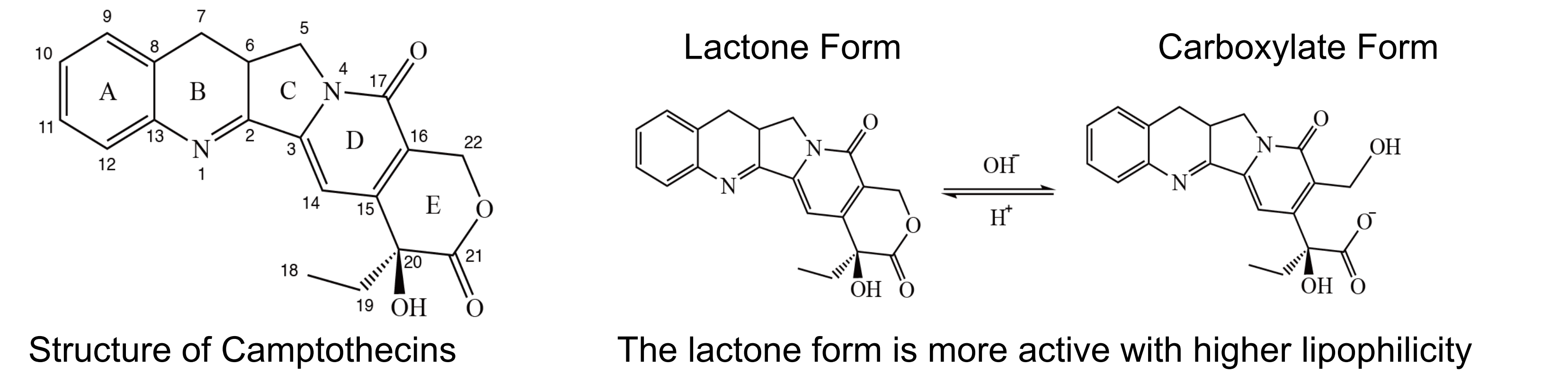


Figure 2. pH-Dependent Structures of Camptothecins (Parent Compound of Exatecan)

Experimental Design

- LBA-based PK assays were established using anti-idiotypic antibody as capture reagent and anti-human IgG antibody as detection reagent for ADC quantification. Standard curve performance and system suitability criteria were applied according to internal bioanalytical procedure.
- Multiple acetic acid (AcOH) acidification conditions were tested by varying acid type, pH, and pre-incubation time. ADC stability under acidification was examined in two ways: (1) incurred PK samples were analyzed for TA**b** under non-acidified conditions (pH ≈ 7.0) and after overnight AcOH treatment (pH ≈ 2.6) followed by neutralization with Tris base to evaluate the impact of acidification towards antibody part of ADC; (2) in vitro spiked high- (HQC, 4000 ng/mL) and low- (LQC, 125 ng/mL) quality control samples were incubated under acidic conditions for 0, 2, 4, 17, 22.5, and 26.5 h, and quantified using a 20 h-acidified standard curve to assess relative recovery over time.
- Incurred PK samples were re-analyzed with optimized assay conditions.

RESULTS

- For incurred PK samples, TA**b** values after overnight acidification remained within –3.1% to 18.8% of the non-acidified condition across early (0.083 h) and late (672 h) time points, indicating no meaningful loss of antibody integrity or impact of the acidification/neutralization procedure on total antibody concentration. (Table 1)

Table 1. Comparison TA**b** behaviors from incurred PK samples between acidification and no-acidification.

Incurred PK Sample ID	Total Antibody Conc. (ng/mL)		%Bias
	Condition 1 No Acidification (pH ≈ 7.0)	Condition 2 Overnight Acidification (HAc/pH ≈ 2.6 + Tris base)	
0.083 h #1	262000	254000	-3.1
0.083 h #2	229000	234000	2.2
672 h #1	11200	13300	18.8
672 h #2	11800	13300	12.7

- The refined acetic acid/Tris acidification procedure had showed *in vitro* that ADC signal progressively increased with incubation time and reached a stable plateau between ~17 and 26.5 h. Under these optimized conditions, application to incurred PK samples restored the expected TA**b** ≥ ADC relationship, yielding TA**b**/ADC ratios >0.90 at both 0.083 h and 672 h. (Figure 3)

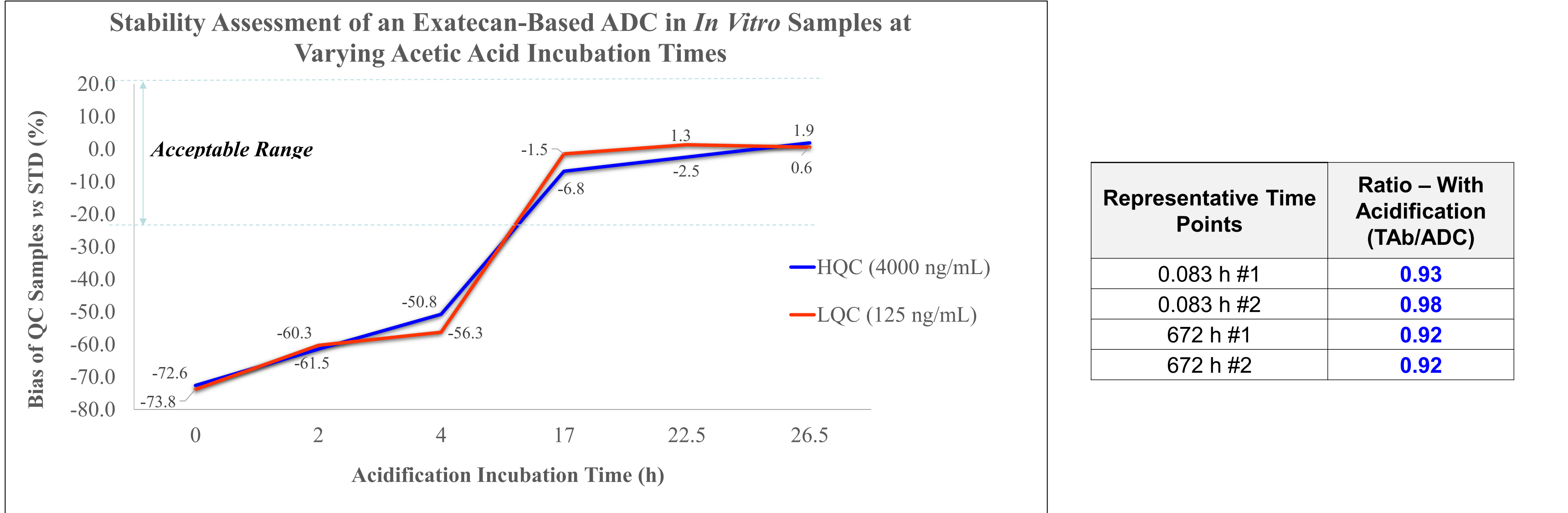


Figure 3. Stability assessment of an Exatecan-Based ADC in *In Vitro* Samples at Varying Acidification Incubation Times

CONCLUSION

- For lactone-containing ADCs, prolonging the acidification step can be critical to reaching speciation equilibrium without compromising stability and should be systematically evaluated when conventional, shorter incubations fail to correct quantitation bias.

REFERENCES

[1] Han, S.; Lim, K.S.; Blackburn, B.J.; Yun, J.; Putnam, C.W.; Bull, D.A.; Won, Y.-W. The Potential of Topoisomerase Inhibitor-Based Antibody–Drug Conjugates. *Pharmaceutics* 2022, 14, 1707.

[2] Zhang Y, Yun X, Ouyang L, Zhang X, Gong L, Qin Q. Development of an ELISA with acidification treatment for an antibody conjugate incorporating Exatecans. *Anal Biochem.* 2024 Jul;690:115530. doi: 10.1016/j.ab.2024.115530. Epub 2024 Apr 2. PMID: 38570023.

