# Antibody-Drug Conjugate (ADC) Quantitation in Human Serum and Innovative Solutions to Stability Challenges

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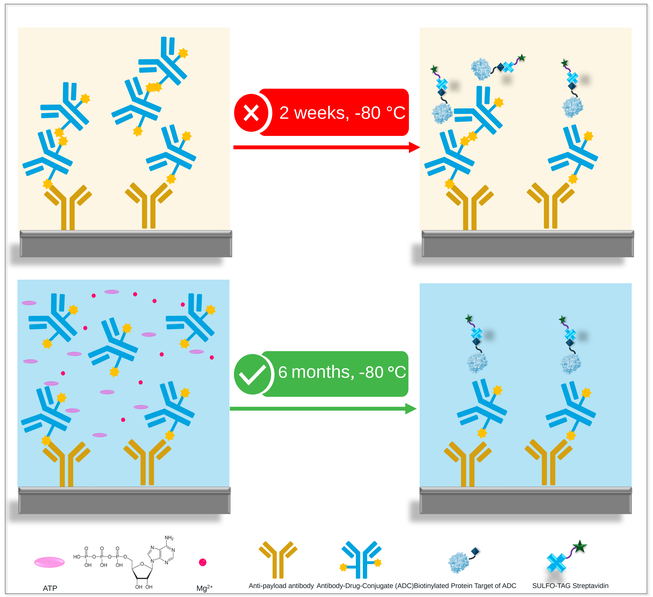


## Introduction

During the development of a bioanalytical method to quantify an ADC in human serum, an unusual stability issue was observed. Stability samples at HQC level (7500 ng/mL) showed a positive bias of around 20%, while samples at LQC level (300 ng/mL) showed a negative bias of around -20%. This indicated potential drug aggregation under assay conditions, possibly due to the modification of the antibody by the linker-payload, which tends to cause ADC aggregation.

Adenosine triphosphate (ATP) and $MgCl_2$, known to prevent and dissolve protein aggregates,[1] were added to the sample diluent at 10 mM each. This reformulation reduced the bias of stability samples at both HQC and LQC levels to within ±20%, while maintaining assay performance parameters such as signal magnitude, curve shape, signal-to-noise ratio, accuracy, precision, and selectivity.

Even with 10 mM ATP and $MgCl_2$, a high shaking speed still failed to stabilize the samples, supporting the aggregation hypothesis and demonstrating the intricate synergistic impact of multiple factors on the apparent stability of the ADC method.

## Method

This bioanalytical method for the quantitative determination of an ADC in human serum is a sandwich-based immunoassay employing electrochemiluminescence (ECL) technology. The capture reagent is an anti-payload antibody, the primary detection reagent is the protein target of the antibody portion of the ADC, and the secondary detection reagent is SULFO-TAG streptavidin. Samples are diluted to a minimum required dilution (MRD) of 1:300 using sample diluent BSA PBST containing 10 mM ATP-Mg. The method has a calibration range of 50 to 15,000 ng/mL and a quantitation range of 100 to 10,000 ng/mL. The calibration standard curve is fitted using a 4-parameter logistic regression model with weighting $1/Y^2$. The responses of Quality Controls (QCs) and test samples are measured and interpolated from the fitted calibration curve.

## Validation Results

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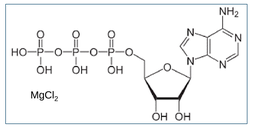
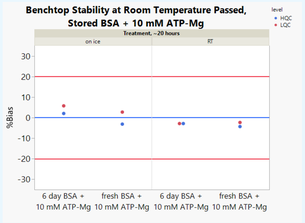
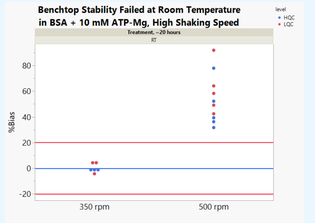
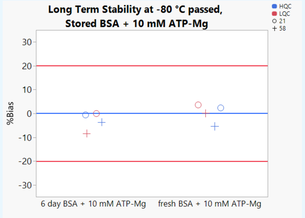
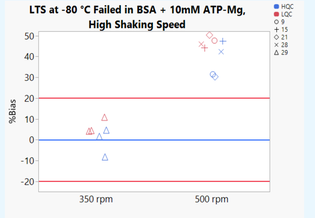
| *Inter-run Precision* | LLOQ QC (100 ng/mL): 6.9%<br>Low QC (300 ng/mL): 8.1%<br>Middle QC (1000 ng/mL): 6.9%<br>High QC (7500 ng/mL): 6.1%<br>ULOQ QC (10,000 ng/mL): 8.3% |
| *Inter-run Accuracy* | LLOQ QC (100 ng/mL): -6.9%<br>Low QC (300 ng/mL): -2.3%<br>Middle QC (1000 ng/mL): -5.2%<br>High QC (7500 ng/mL): -3.3%<br>ULOQ QC (10,000 ng/mL): -5% |
| *Dilution Linearity* | Analysis of 960,000 ng/mL of ADC in human serum samples at dilution factors up to 3000 were acceptable. No prozone effect was observed up to 960,000 ng/mL. |
| *Selectivity (Normal Healthy)* | 10 of 10 unspiked individual lots found to be BLQ<br>10 of 10 individual lots spiked at LLOQ and High QC level met acceptance criteria |
| *Selectivity (Disease State)* | 5 of 5 unspiked individual lots found to be BLQ<br>5 of 5 individual lots spiked at LLOQ and High QC level met acceptance criteria |
| *Hemolyzed Samples* | Unspiked hemolyzed pool found to be BLQ<br>Spiked pool at LLOQ met acceptance criteria, while the spiked pool at the High QC level failed to meet acceptance criteria |
| *Lipemic Samples* | Unspiked lipemic pool found to be BLQ<br>Spiked pool at LLOQ and High QC level met acceptance criteria |
| *Robustness* | Maximum and minimum incubation times, incubation at room temperature, two lots of secondary detection reagent, 7 day storage at 2-8°C for sample diluent, and 3 day coating at 2-8°C were tested and met acceptance criteria |
| *Benchtop Stability*<br>*Freeze/Thaw Stability*<br>*Long-Term Stability* | Up to 21.0 hours at room temperature<br>Up to 5 cycles at -80°C<br>Up to 29 days at -20°C and 190 days at -80°C |
| *Specificity* | No interference with the detection of ADC in the unspiked, LLOQ-level spiked, and ULOQ-level spiked serum samples from the unconjugated antibody of ADC up to 200 µg/mL. |

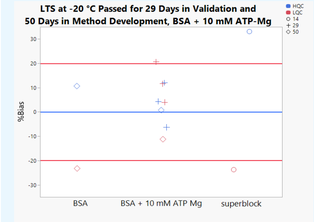


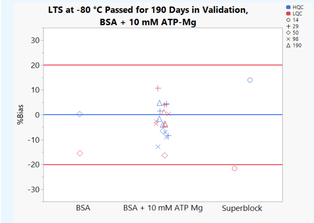



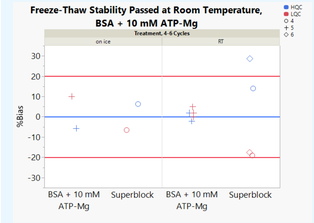



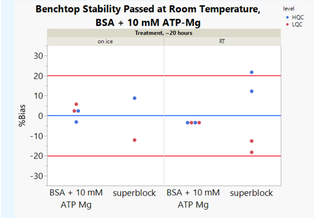




## Conclusions

A quantitative ADC method exhibited potential stability issues in benchtop and freeze-thaw evaluations. The long-term stability failed at the two-week time point at -20°C or -80°C storage. By supplementing the sample diluent with **10 mM ATP-Mg,** the stability issues were successfully resolved. Benchtop stability for up to 21 hours at room temperature and freeze-thaw stability for up to 5 cycles at room temperature were established. Long-term stability has been established for up to 29 days at -20°C and **190 days at -80°C** with on-going storage. The updated method has been validated to support quantitating the ADC in clinical trials. This approach can serve as a potential option for ADCs or therapeutic protein drugs that exhibit similar stability issues.

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**Reference: 1. Patel et al., Science 356, 753–756 (2017)**
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