Implementing Design of Experiment (DOE) in ADA Optimization for Targeted Therapeutics

POSTER #B013

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INTRODUCTION AND PURPOSE

In addition to Enzyme Replacement Therapies (ERTs), new modalities such as adeno-associated virus (AAV)-vectored gene therapies have shown great promise in the treatment of rare genetic diseases. The targeted activity of these therapeutics means that bioanalytical assays often need to be developed in unique matrices, such as cerebrospinal fluid (CSF), which may be limited in volume and contain interfering factors that complicate assay development. Therefore, approaches that shorten development and reduce matrix requirements are critical.

Design of Experiment (DOE) is a statistical approach used to shorten the time involved in developing bioanalytical assays by testing the relationship between multiple factors simultaneously. Herein, we detail two case studies where DOE was implemented to streamline anti-drug antibody (ADA) assay development in CSF. The first case study details how DOE was used to design an ADA assay for AAV-based capsid with optimized sensitivity. The second case study applies DOE to developing a drug tolerant ADA assay for an ERT for a lysosomal storage disease with drug delivered directly to the central nervous system (CNS).

CASE STUDY #2 – DOA APPROACH TO MAXIMIZE DRUG TOLERANCE FOR AN ERT ADA ASSAY

METHODS: This assay was a bridging ECL method where samples were pre-incubated with a master mix containing both biotinylated drug for capture and ruthenium labeled drug for detection prior to addition to a streptavidin coated MSD plate.

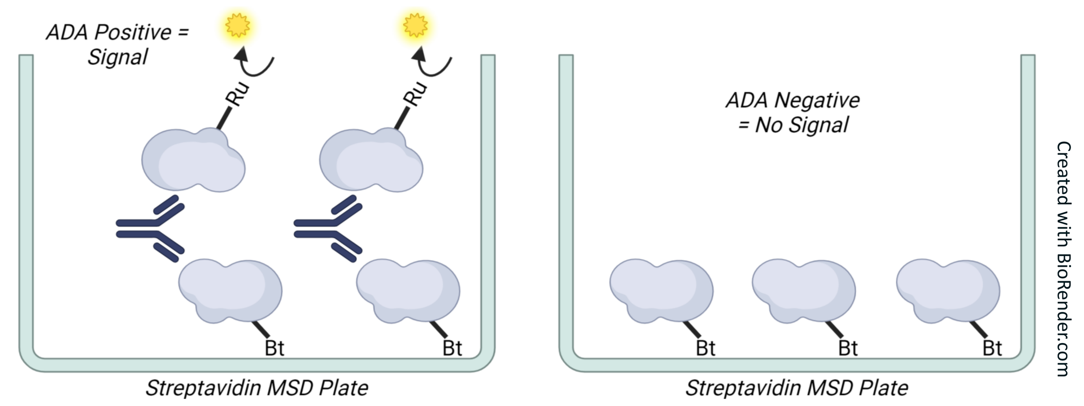

Figure 6. Method Schematic for ERT ADA assay. ADA present in human CSF samples bridge the Ruthenylated drug to the Biotinylated Drug, resulting in a signal.

RESULTS: In this case study, a critical aspect of designing the ADA assay for a lysosomal storage disease targeted ERT was maximizing drug tolerance (DT) since the ERT was expected to be present at high levels in CSF. Preliminary experiments demonstrated that adding an acid dissociation step to the assay had detrimental effects on assay performance. Altering concentrations of the labelled drug and the MRD had the greatest impact on drug tolerance in the original format. Therefore, a full factorial (2x3) DOE approach was used to test the concentration of labelled drug (LD) in the reaction solution at three equimolar concentrations (0.5 µg/mL, 1.0 µg/mL, and 2.0 µg/mL) and at 3 MRD levels (1:2, 1:4, and 1:8). Each condition was tested with 3 samples: NC (unspiked CSF pool), positive control (31.25 ng/mL SPC spiked in CSF pool), and DT condition (500 ng/mL SPC and 1.25 µg/mL of free drug in pooled CSF). DT was optimal at 1.0 µg/mL LD and MRD 1:4 (Figure 7). MRD was further optimized at 1:3 and the final DT under these conditions was 20 µg/mL (Figure 8).

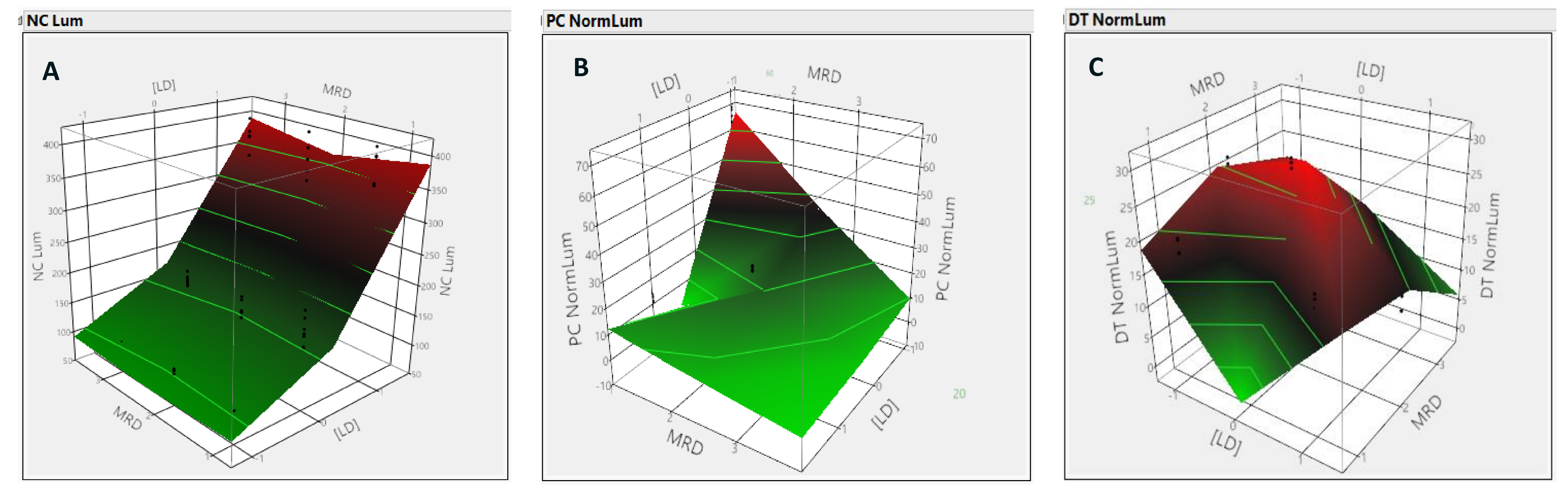

Figure 7: Response surface comparison of 3 concentrations of labeled drug and 3 different MRD levels across NC (A), PC (B), and Drug Tolerance (C) samples.

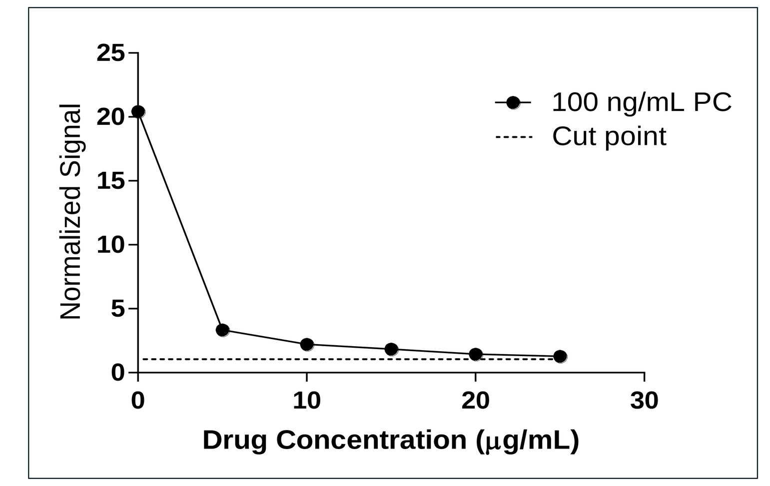

Figure 8. Drug Tolerance at MRD 1:3

CASE STUDY #1 – DOA APPROACH TO IMPROVE SENSITIVITY IN AN ADA ASSAY FOR AN AAV-BASED CAPSID

METHODS: This ADA assay was a sequential bridging ECL method. Briefly, MSD plates were coated with unlabeled AAV for capture, followed by blocking, then incubation with human CSF and detection with a ruthenium labeled AAV (Ru-AAV) and MSD Read Buffer T. Increase signal within the well correlates with the increased presence of ADA.

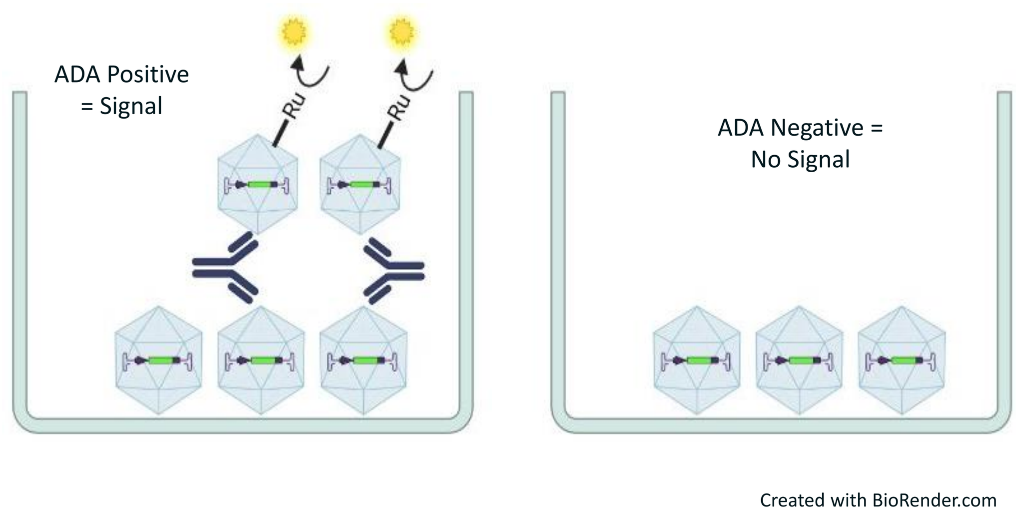

Figure 1. Method Schematic for AAV ADA assay. ADA present in human CSF samples binds to the AAV on the plate and is detected with Ru-AAV, resulting in a signal.

RESULTS: In this case study, experiments were designed and performed in which to evaluate the impact of blocking reagents and MRD against sensitivity and drug tolerance in a full factorial (2x3) DOE approach. BSAs from two different vendors (data from only one vendor shown) and casein were evaluated as blocking reagents with three concentrations each against MRDs of 1:8, 1:16, or 1:32 (Figure 2). Each condition was tested in NC (unspiked CSF pool), positive control (SPC, 100ng/mL in CSF pool), and with 500 ng/mL AAV9 Capsid spiked with SPC in CSF pool to assess drug tolerance. As shown in Figure 2A, the [BSA] had a greater impact on signal than MRD and the maximum drug tolerance was achieved with an MRD of 1:8 in combination with 2% BSA (Figure 2C). Therefore, 2% BSA blocker was further evaluated with MRD by testing the variance of 10 individual CSF samples at 1:8 and 1:16 MRD. Mean luminescence values were similar for all 10 samples with the MRD of 1:16 showing less variance (Figure 3). %CV for individual samples at MRD of 1:8 was 5.2%, further indicating this MRD was appropriate. The performance of the assay under the optimized conditions is shown in Figure 4-5. At MRD 1:16, the final assay sensitivity was 7.8 ng/mL with a drug tolerance of 100 ng/mL.

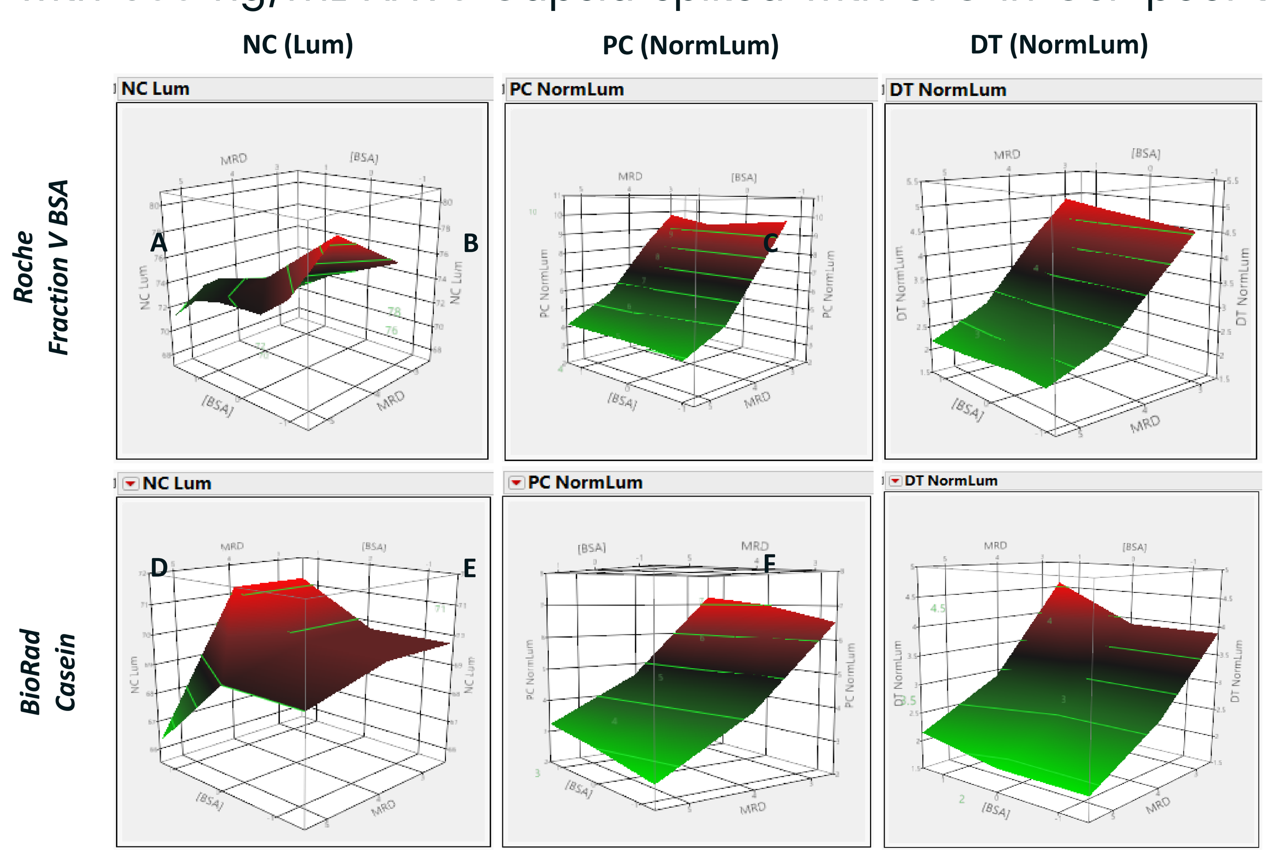

Figure 2: Response surface comparison of 2 blockers on MRD. BSA Blocker was tested at 2%, 1%, 0.5%; casein blocker tested at 1%, 0.5%, 0.25%.

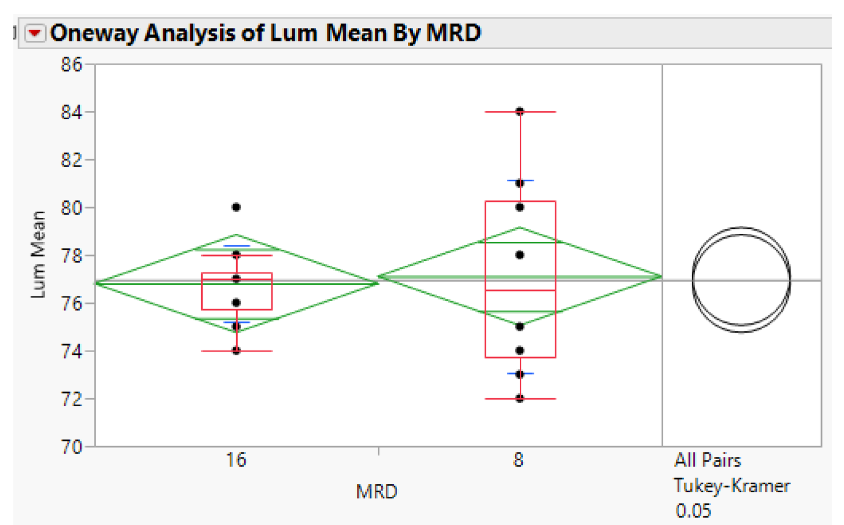

Figure 3: MRD Assessment in Roche Buffer.

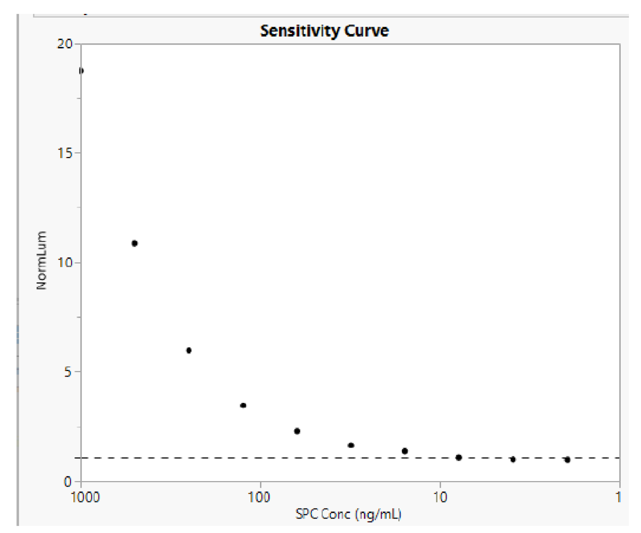

Figure 4: Sensitivity Curve at MRD 1:16

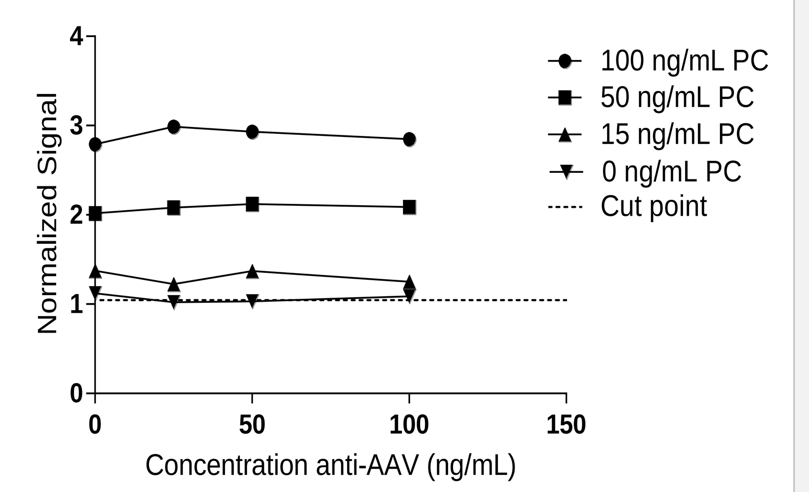

Figure 5: DT at MRD 1:16

CONCLUSIONS

- In the first case study, a 2 factor, 3 level DOE identified the best conditions that minimized matrix interference and maximized sensitivity. This led to an identifying conditions for an improved assays sensitivity of 7ng/mL with acceptable selectivity in individual CSF samples.
- In the second case study, 2 factor, 3 level DOE facilitated the selection of conditions with acceptable drug tolerance of 20µg/mL at the SPC concentrations of 100ng/mL.
- Using DOE for CSF enabled the testing of multiple conditions while minimizing the amount of matrix used.
- In both cases, DOE was successfully implemented, reduced development by approximately one week to mitigate matrix interference issues, and improved assay performance in CSF.