The challenging road to success: from ELISA to Gyrolab™ via MSD

Case Study

Envigo were asked by a client to develop and validate a PK/TK assay for a monoclonal antibody used to treat cancer at levels as low as 10 ng/ml in cyno samples. The company first transferred the assay from ELISA to the MSD platform before finally transferring it to Gyrolab™ system, where the development and validation work was completed. Sarah Geen described the challenges encountered throughout this process and the strategies used to overcome them at the European Gyrolab Seminar in Amsterdam in June 2016.

Envigo encountered a number of issues suggesting that the homogenous ELISA proposed by the client might not be suitable for the study. For example, sensitivity was 100 ng/mL, which was not sufficient for the samples to be analyzed, and a hook effect was observed at 1 µg/mL which was not optimal with expected C_{max} values of 800 µg/mL. Envigo thus investigated alternatives to the proposed ELISA format.

Envigo routinely use a Design of Experiments (DoE) approach when developing and optimizing assays. The Design Expert® software helped identify optimum conditions by analyzing various capture and detection concentrations as well as different antibody combinations. DoE on the MSD format revealed the following optimum conditions for a biotin-anti-drug antibody and a BD sulfo-tagged mouse anti-human IgG antibody: capture; 6 µg/mL, detection; 3.8 µg/mL, MRD; 1 in 4, predicted range; 10 ng/mL to 1000 ng/mL. Although this was a real step forward, the assay was still not sensitive enough. The move to Gyrolab system revealed huge differences in the DoE process between the two platforms.

Superior DoE on Gyrolab system

MSD requires about four plates with about eight different experiments on each. Gyrolab system, on the other hand, needs just two CDs and automatically mixes all the different combinations of capture and detection reagents. Two full working days on MSD is transformed into about three hours work on the Gyrolab instrument. Furthermore, the results obtained were better: capture; 100 µg/mL (using 5 µl), detection; 166.7 nM, MRD; 1 in 2, predicted range; 10 ng/mL to 2560 ng/mL. This latter parameter thus has a much-extended range on Gyrolab compared with MSD. Gyrolab also gave a superior precision profile (Fig. 1).

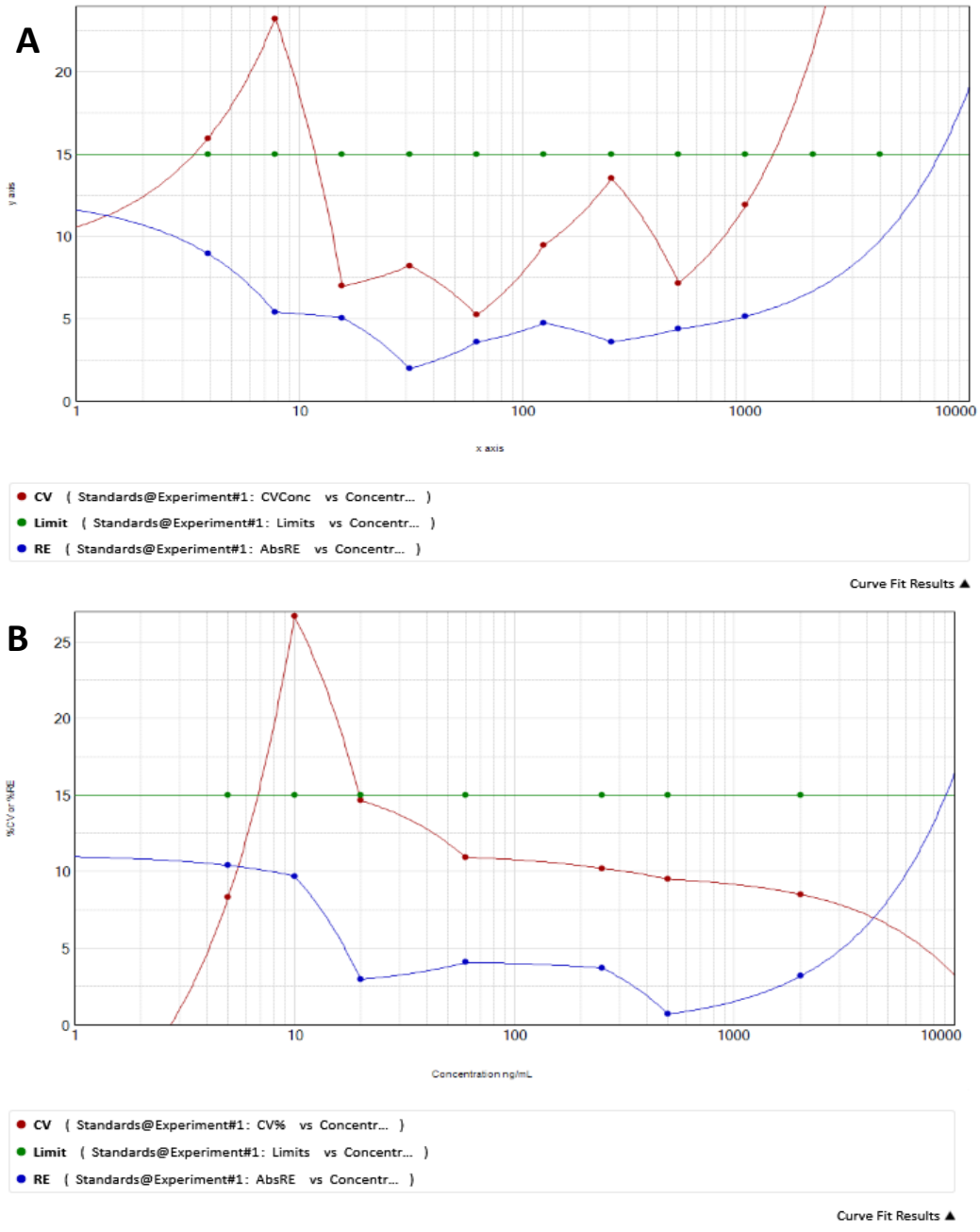

Fig. 1. *The precision profile obtained on Gyrolab system (A) was superior to that obtained on the MSD platform (B).*

Gyrolab assay met selectivity acceptance criteria

Sensitivity and matrix effects were encountered throughout the study and the origin of the serum was found to play an important role. Vietnamese cyno serum gave a much cleaner signal compared with Mauritius cyno serum and the former was ultimately used in validation and sample analysis. Even so, MSD selectivity data on Vietnamese cyno individuals failed to meet set acceptance criteria, with only three or four of ten samples being good enough to pass. In contrast, seven of ten lows and nine of ten highs met the criteria on Gyrolab system (Fig. 2).

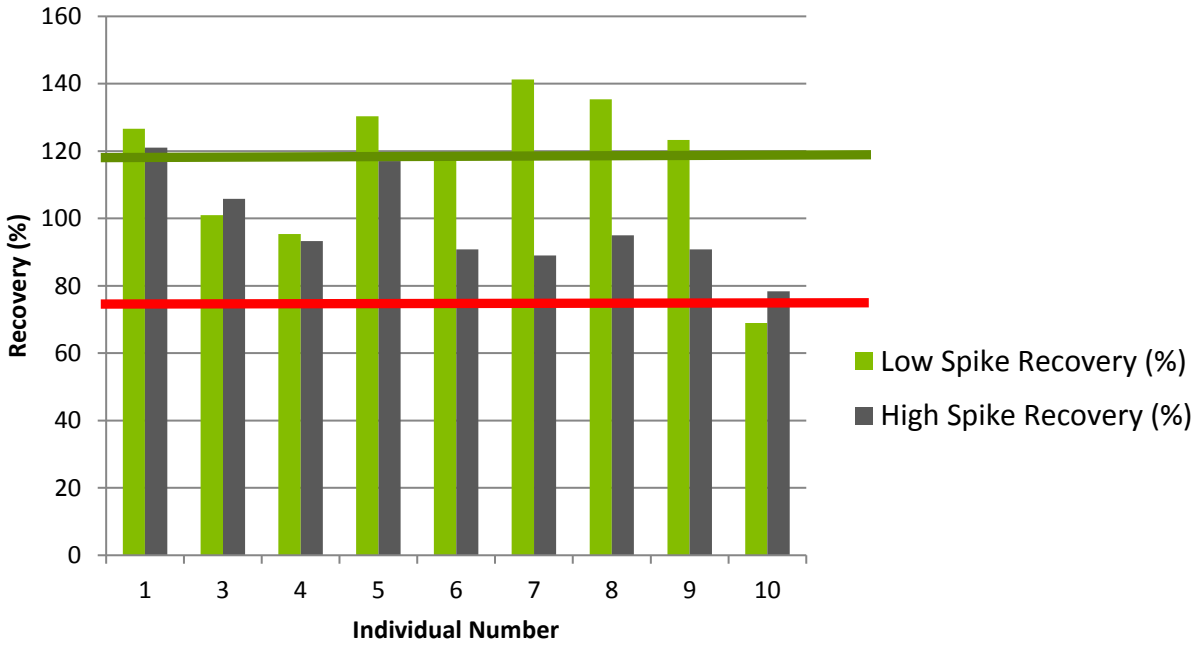

Fig. 2. *Selectivity results on Gyrolab system meet set acceptance criteria.*

Problems with the sticky drug

Sarah and the team were concerned that the drug was sticking to pipette tips or plastic tubes. For example, they noted the precision profile signal was decreasing across each concentration, as well as high CVs, which is obviously a significant issue. They switched to Gyrolab Wash Buffer pH 11 to ensure that the needles were washed effectively. Using a detergent within the Rexxip A max sample dilution buffer helped, as did using non-stick plastic-ware for diluting samples and storing QC samples and standards. These measures alleviated the problem.

PK and TK studies completed the assay investigation

PK and TK sample analysis studies were both performed on Gyrolab Bioaffy 1000 CD with quite a large difference in the doses; with TK, the highest dose was 40 mg/kg/occasion.

The PK results were at first rather strange. Many were below the Level of Quantification (BLQ), so they were concerned that perhaps the drug had a very short half-life and had been cleared quite quickly, or maybe that the assay was simply not sensitive enough. However, ADA studies with the same samples returned high levels of ADAs for the drug. A typical PK profile shows the Cmax slowly dropping off over time but still detectable, but in this case, it is likely that the ADA's immediately bind to the drug and that is why there were only detectable levels of drug for the first few time points.

Why is Gyrolab system better for sample analysis?

Over 800 samples had to be analyzed in the TK study. Timelines were tight and there was no time for error or many repeats. Whereas MSD requires two days for 60 to 90 samples, Gyrolab handles 240 samples in just one day. What Envigo could accomplish on Gyrolab in one week would have taken four or five weeks on the MSD platform.

In conclusion, this was a very challenging assignment. By offering increased sensitivity and range, less matrix effects, speedy development and high sample turnover, Gyrolab proved to be the superior platform for completing the assay development, validation and sample analysis.