Optimizing a PK Assay for Reliable Data in Clinical Studies

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Introduction

Ligand binding assays are important tools for quantifying biopharmaceuticals, biomarkers, and evaluating immunogenicity in both preclinical and clinical research. The results derived from these assays often guide critical decisions, and it is therefore crucial that the data are reliable.

This poster highlights some key aspects to consider when improving a ligand-binding assay. A pharmacokinetic (PK) assay, transferred to Mercodia from a sponsor, encountered challenges during its initial implementation in a first-in-human clinical study. A re-optimization was performed in an iterative way with stepwise identification of the most crucial parameters to ensure a cost-efficient optimization.

Solving the issue with edge effects

A PK assay was transferred to Mercodia and validated. The assay was then used to analyze samples in clinical phase 1. However, during the long study, a new lot of commercial coated plates was required due to the short expiration date of the previous lot. Unfortunately, several runs using the new plate lot were not approved due to inaccurate QC results. Upon investigation, it was discovered that the new plate lot had edge effects and high CV% (as shown in Figure 1). This resulted in the measured concentrations being dependent on the position in the plate.

Short-term solution

An urgent solution was required to continue the phase 1 clinical study. The decision was made to exclude the wells at the edges, resulting in fewer reruns and more reliable data within the given timeline. However, this also meant that a smaller number of samples could be analyzed per plate, prompting the need for a long-term solution.

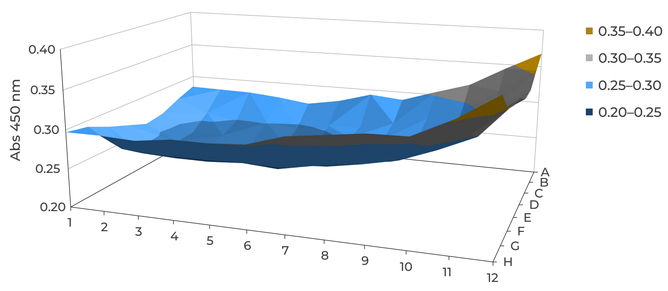

Figure 1. External commercial coated plate, CV%=13.1%. Homogeneity was investigated with one QC sample in all 96 wells.

Long-term solution

As a long-term solution to the issue with edge effects, Mercodia-coated plates were introduced as an alternative. The coating was done with in-house protocols that built on >30 years' experience of coating plates without edge effects in our kit production. The plates were validated and found to exhibit good precision (Figure 2), accuracy, and long shelf time stability (at least 3 years). The results obtained using these plates during the clinical phase 2 study were reliable and cost-effective, requiring fewer runs and no reruns.

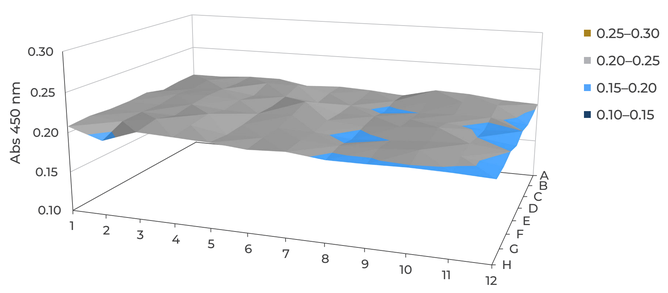

Figure 2. Mercodia in-house coated plate, CV%=2.4%. Homogeneity was investigated with one QC sample in all 96 wells.

Solving the issue of false positives in pre-dose samples

Selectivity was validated prior to the study by analyzing plasma samples from 10 healthy individuals. The concentrations obtained were below the lower limit of quantification (LLOQ), fulfilling the acceptance criteria. During the sample analysis, it was found that up to 25% of the pre-dose samples had measurable concentrations above LLOQ. No measurable concentrations are expected in pre-dose samples before the drug is administered.

Short-term solution

An urgent solution was required for the non-specific response to continue the clinical study. A blank subtraction was evaluated and validated to correct the response in post-dose samples, using the concentration in each individual pre-dose sample. This method provided reliable data during the clinical phase 1 study. However, a more permanent solution would be to reduce the non-specific binding.

Long-term solu3tion

In ligand binding assays non-specific binding from e.g., heterophilic antibodies often needs to be reduced with blocking reagents in buffers. A Mercodia proprietary conjugate dilution buffer with blocking reagents reduced the non-specific binding seen in pre-dose samples (Table 1) without reducing the specific binding (Table 2). The assay was successfully validated before use in the clinical phase 2 study.

Table 1. Measured concentrations in problematic pre-dose samples. The LLOQ in the assay was 0.1 ng/mL.

Pre-dose samples	Original method	Mercodia coated plate and proprietary conjugate buffer
	Conc. (ng/mL)	Conc. (ng/mL)
1	0.346	< LLOQ
2	< LLOQ	< LLOQ
3	0.270	< LLOQ
4	< LLOQ	< LLOQ
5	0.132	< LLOQ
6	0.101	< LLOQ
7	0.384	< LLOQ

Table 2. Measured concentration of QC samples

QC samples	Nominal	Original method		Mercodia coated plate and proprietary conjugate buffer	
	Conc. (ng/mL)	Conc. (ng/mL)	Accuracy	Conc. (ng/mL)	Accuracy
L	0.25	0.20	80%	0.25	101%
M	1.30	1.31	101%	1.29	99%
H	6.50	6.94	107%	6.71	103%

Ease of use

The original protocol involved serially diluting the calibrators in several steps from a high-concentration stock solution before each run. However, to make the assay more robust and reduce the risk of differences in the dilution of the calibration curve in different runs, the possibility of using pre-made frozen calibrators was explored. As per ICH M10 recommendations, frozen standards can be used within their defined period of stability. Validation showed that accuracy, precision, freeze/thaw, bench top and long terms stability of the frozen calibrators were within acceptance criteria, and they could thus be used in the sample analysis.

Additionally, to increase the ease of use of the assay, the reagent solutions were color-coded (as shown in Figure 3).

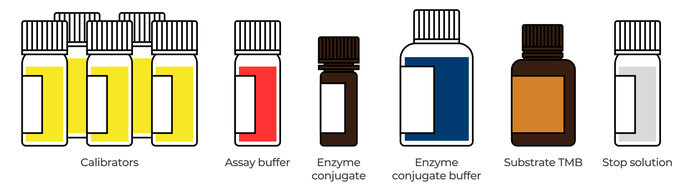

Figure 3. Color-coded reagents that improve the pipetting to the plate.

Life cycle management

Effective life cycle management is crucial for obtaining reliable and robust data throughout the various phases of drug development, which often extend over long periods of time. This may include characterizing the critical reagents during assay development, certified production of kits, maintaining control of assay quality through standard procedures such as validation and lot-to-lot comparison, stability studies of the kit, and developing processes for corrective and preventive actions (CAPA) (as shown in Figure 4).

The re-optimized assay was incorporated into selected parts of the Mercodia life cycle management program and was used in multiple studies, demonstrating excellent reproducibility over time.

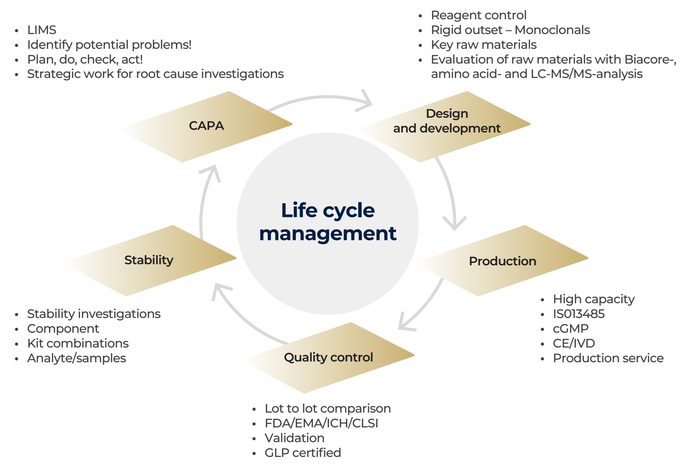

Figure 4. Life cycle management

Conclusion

An assay transferred to Mercodia underwent re-optimization, resulting in significant improvements.

The changes made to the assay:

- minimized the risk of false positives in the relevant matrix, ensuring reliable data.
- enhanced precision and accuracy (eliminating plate edge effect), giving valid data.
- resulted in fewer runs and re-runs being required, making the overall process cost- and time-effective.

The re-optimized assay was fully validated and successfully used for sample analysis in phase 1 and phase 2 studies.

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