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CURRENT STRATEGIES FOR USING COMMERCIAL LBA KIT IN REGULATED BIOANALYSIS

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PURPOSE

Pharmacokinetic/Toxicokinetic (PK/TK) bioanalysis of a biological compound by ligand binding assay (LBA) typically requires developing a specific and tailor-made method, entailing extensive generation times for drug-specific critical reagents, such as antibodies. Commercial kits are potential solutions that contain ready-to-use assay plates and critical reagents with recommended procedures, which can significantly accelerate the development of an LBA method. However, as they lack the required specification criteria and standardization in critical reagent characterization to support regulated pharmacokinetic (PK) and pharmacodynamic (PD) studies, repurposing must be made to align the method with regulatory study requirements. This may involve updating the reference standard, the standard curve range, and the matrices. Further challenges pertain to limited quantities of specific kit lots that can impact long-term planning as clinical studies require. Therefore, kit lot-to-lot qualification needs to be properly planned and executed. We provide examples of such challenges and mitigation strategies to repurpose commercial kits for regulated PK bioanalysis.

OBJECTIVE(S)

This poster describes challenges observed with kits in three independent studies:

1. Kit repurposing to improve the coefficient of variation (%C.V.) robustness
2. Kit repurposing to minimize matrix interferences
3. Kit lot-to-lot qualifications

METHOD(S)

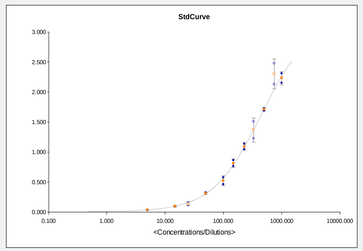
A brief summary of the kit-based methods in question:

- **Study 1:** Method to quantify a recombinant protein in a phase 1 clinical study with healthy subjects. Reference standard from the sponsor was used with the kit-supplied instructions. Method lacked robust %C.V. between replicates, signal plateau not reached due to likely issue with color development.
- **Study 2:** Method to quantify a monoclonal antibody (mAb) in rat serum. Reference standard from the sponsor was used with the kit-supplied instructions. Significant issues with matrix effects observed.
- **Study 3:** Method to quantify a biomarker. After successful method development (MD), method validation was initiated with a different lot due to the unavailability of the lot used in MD. New kit lot had %C.V. Issues, as later confirmed by the manufacturer.

RESULTS

STUDY 1 (PK BIOANALYSIS)

Performance with kit procedure:

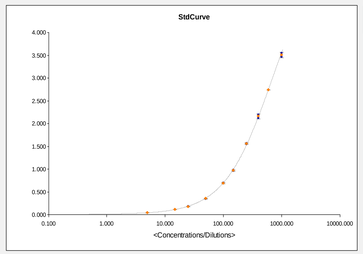


Sample ID	Nominal	Signal	Mean Conc.	C.V .(%)	% Recovery
BLK	0	0.009 0.008	NA	NA	NA
STD1	5	0.04 0.036	4.998	10.5	100.0
STD2	15	0.105 0.093	14.951	9.4	99.7
STD3	25	*0.165* *0.119*	*UCV	*UCV	*UCV
STD4	50	0.331 0.304	52.265	6.6	104.5
STD5	100	0.583 0.467	91.901	18	91.9
STD6	150	0.867 0.767	157.522	11.2	105.0
STD7	230	1.144 1.053	236.188	8.6	102.7
STD8	330	*1.230* *1.513*	*UCV	*UCV	*UCV
STD9	500	1.696 1.734	503.926	3.2	100.8
STD10	750	*2.131* *2.482*	*UCV	*UCV	*UCV
STD11	1000	2.158 2.312	985.469	15.2	98.5

- Unacceptable %C.V. observed
- Signal plateau not reached
- Color development issue suspected

Method was further optimized:

- Sample loading volume decreased from 100 to 50 µL.
- Stop solution volume and concentration updated from 50 µL of 2 N to 100 µL of 1 N.
- Substrate incubation time decreased from 20 to a 15-18 min range.
- Shaking step added following stop solution addition.



Sample ID	Nominal	Signal	Conc. Mean	C.V .(%)	% Recovery
BLK	0	0.01 0.016	0.672	NA	NA
STD1	5	0.042 0.042	4.953	0.4	99.1
STD2	15	0.118 0.118	15.72	0.3	104.8
STD3	25	0.184 0.176	24.364	3.2	97.5
STD4	50	0.353 0.358	49.211	1	98.4
STD5	100	0.706 0.687	100.482	2.1	100.5
STD6	150	0.964 0.984	146.112	1.7	97.4
STD7	250	1.552 1.573	259.23	1.2	103.7
STD8	400	2.127 2.202	407.384	3.7	101.8
STD9	600	2.741 2.745	597.46	0.2	99.6
STD10	1000	3.554 3.47	979.321	3.9	97.9

%C.V. and %recovery are acceptable.

Conclusions:

- Kit-supplied stop solution was swapped for an in-house preparation.
- Additional updates to the kit-recommended procedure improved assay performance significantly.

STUDY 2 (PK BIOANALYSIS)

Performance with kit procedure:

Kit method repurposed during method development to adapt to mAb preclinical study requirements:

- Reference standard used from the sponsor.
- Suggested curve range increased from 0.1 to 10 ng/mL to 10 to 1000 ng/mL to accommodate matrix effects and an estimated C_{max} of 54000 ng/mL

Matrix Lot	Gender	Neat	LLOQ QC (ng/mL) 10.0		HQC (ng/mL) 700		Lot Acceptance
		Mean Conc. (ng/mL)	Mean Conc. (ng/mL)	%Bias	Mean Conc. (ng/mL)	%Bias	(Y/N)
RAT488189	Male	<LLOQ	9.35	-6.5	586	-16.3	Y
RAT488190	Male	<LLOQ	5.66	-43.4	530	-24.3	N
RAT488191	Male	<LLOQ	9.00	-10.0	656	-6.3	Y
RAT488192	Male	<LLOQ	10.2	2.0	513	-26.7	N
RAT488193	Male	<LLOQ	9.60	-4.0	660	-5.7	Y
RAT488199	Female	<LLOQ	6.60	-34.0	545	-22.1	N
RAT488200	Female	<LLOQ	9.55	-4.5	527	-24.7	N
RAT488201	Female	<LLOQ	5.37	-46.3	UCV	NA	N
RAT488202	Female	<LLOQ	UCV	NA	535	-23.6	N
RAT488203	Female	<LLOQ	UCV	NA	336	-52.0	N

Matrix interference from individual matrix lots observed during validation.

- Under-recovery at lower limit of quantitation (LLOQ), quality control (QC), and high QC (HQC) levels.
- Unacceptable %C.V. [upper critical value (UCV)].

Method was further optimized:

- Minimum required dilution increased from 50- to 100-fold.
- Kit supplied blocking buffer now filtered to reduce UCV, arising from precipitates observed in the plates following blocking.
- LLOQ raised from 10.0 to 50.0 ng/mL.
- Color development monitored prior to adding stop solution.

Performance following repurposing:

Matrix Lot	Gender	Neat	LLOQ QC (ng/mL) 30.0		HQC (ng/mL) 700.0		Lot Acceptance
		Mean Conc. (ng/mL)	Mean Conc. (ng/mL)	%Bias	Mean Conc. (ng/mL)	%Bias	(Y/N)
RAT497751	Male	<LLOQ	27.7	-7.8	681	-2.7	Y
RAT497752	Male	<LLOQ	29.7	-1.0	674	-3.7	Y
RAT497753	Male	<LLOQ	33.6	12.0	659	-5.9	Y
RAT497761	Female	<LLOQ	26.3	-12.3	623	-11.0	Y
RAT497762	Female	<LLOQ	23.4	-22.2	584	-16.6	Y
RAT497763	Female	<LLOQ	27.2	-9.3	628	-10.3	Y

%C.V. and %recovery in acceptable range.

Conclusions:

- Filtration of the kit-supplied blocking buffer aided in reducing UCVs.
- Increasing the minimum required dilution (MRD) and the curve LLOQ mitigated matrix interferences.

STUDY 3 (BIOMARKER ANALYSIS)

Kit-based method developed for a biomarker quantification:

- Multiple kit lots used for method development with no issues.
- Method validation initiated a few months later due to other delays.
- Original kit lots unavailable, therefore the validation was initiated with a new kit lot that was successfully qualified.
- However, multiple failed evaluations observed with the new kit lot.

Run ID	Run Status	Run Description	LLOQ PQC (0.500 pg/mL)	QC1 (1.50 pg/mL)	PQC1 (1.50 pg/mL)	PQC2 (6.25 pg/mL)	PQC3 (9.38 pg/mL)	ULOQ PQC (12.5 pg/mL)
9	Accepted	Endogenous Determination	NA	NA	!!1.19	5.72	8.54	NA
			NA	NA	1.38	5.84	9.64	NA
10	Accepted	Precision 1 (proxy)	*UCV	NA	*UCV	5.38	8.38	11.7
			0.576	NA	1.39	*UCV	9.36	11.3
			0.449	NA	1.43	5.91	9.50	13.8
11	Rejected	Precision 2 (proxy)	NA	NA	1.38	5.58	9.01	!16.7
			0.616	NA	1.50	!4.97	7.55	11.1
			!0.643	NA	1.49	6.23	8.85	!16.9
12	Rejected	Precision 3	!0.359	0.976	1.25	5.18	10.6	12.1
			0.443	!0.825	1.22	!4.53	!6.74	12.2
			0.439	0.949	1.33	6.00	9.46	14.4
13	Rejected	Precision 4	0.464	1.01	!1.09	5.31	8.69	11.8
			0.413	1.28	1.31	5.23	7.92	11.0
			0.499	0.999	1.31	5.12	!7.24	10.1
			!0.288	1.10	1.21	5.45	8.03	12.2
			0.418	!1.33	1.39	5.23	8.39	11.2

Conclusions:

An investigation and a retrospective review of all data revealed:

- 75% of the runs with the new lot had > 2 UCV samples.
- 10% of the runs with the old lot had > 2 UCV samples.
- Manufacturer confirmed the faulty lot after internal inter-lot testing.
- Extensive qualification required for kit lots, as one successful run may not identify potential issues.

CONCLUSIONS

- Commercial kits can greatly accelerate MD for PK/TK bioanalyses to meet timelines, especially when drug-specific reagents are unavailable.
- Potential challenges should be kept in mind in order to repurpose the kits for regulated bioanalysis.
- Long-term planning is imperative due to limitations with the available quantity of kit lots, as lot-to-lot variability can impede progression.

ALTASCIENCES