Singlicate analysis applied to pharmacokinetic ligand binding assays: an overview of benefits and limitations

Experience ICON

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Singlicate LBA analysis

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White Paper

Bioanalysis

European Bioanalysis Forum recommendation on singlicate analysis for ligand binding assays: time for a new mindset

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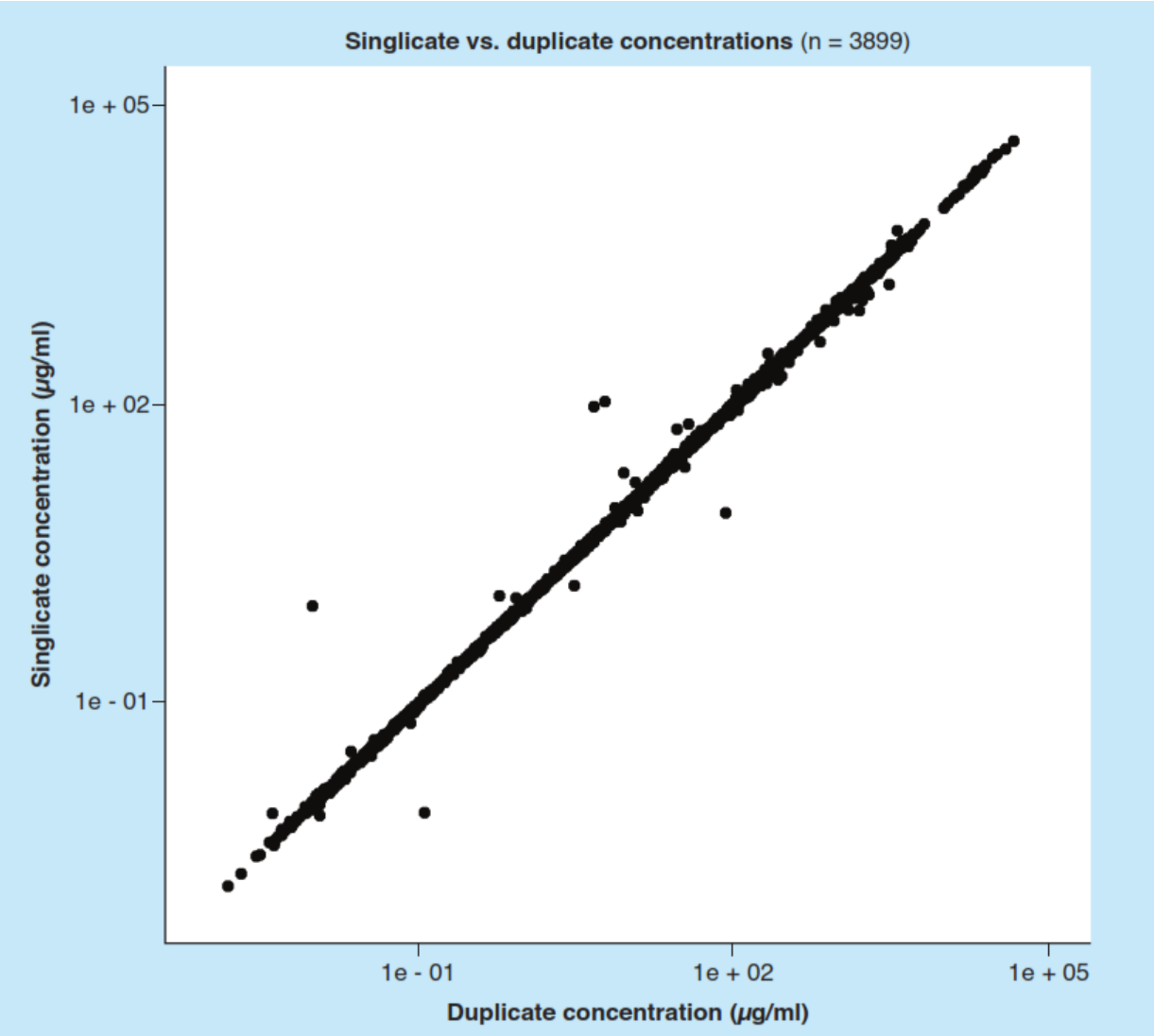
Fourteen studies, three species, three molecule types and three assay platforms

General conclusions from publication:

- TK and PK data show no notable impact on results for C_{max} , AUC and half-life between singlicate and duplicate analyses
- Decision to perform singlicate analysis should be based on validation data
- ISR may be performed to confirm correctness of singlicate analysis

Regression analysis singlicate vs duplicate data



Whole dataset comparison

- 14 studies
  - N = 3899
  - 5 points did not correspond with line (equates to 0.13%)

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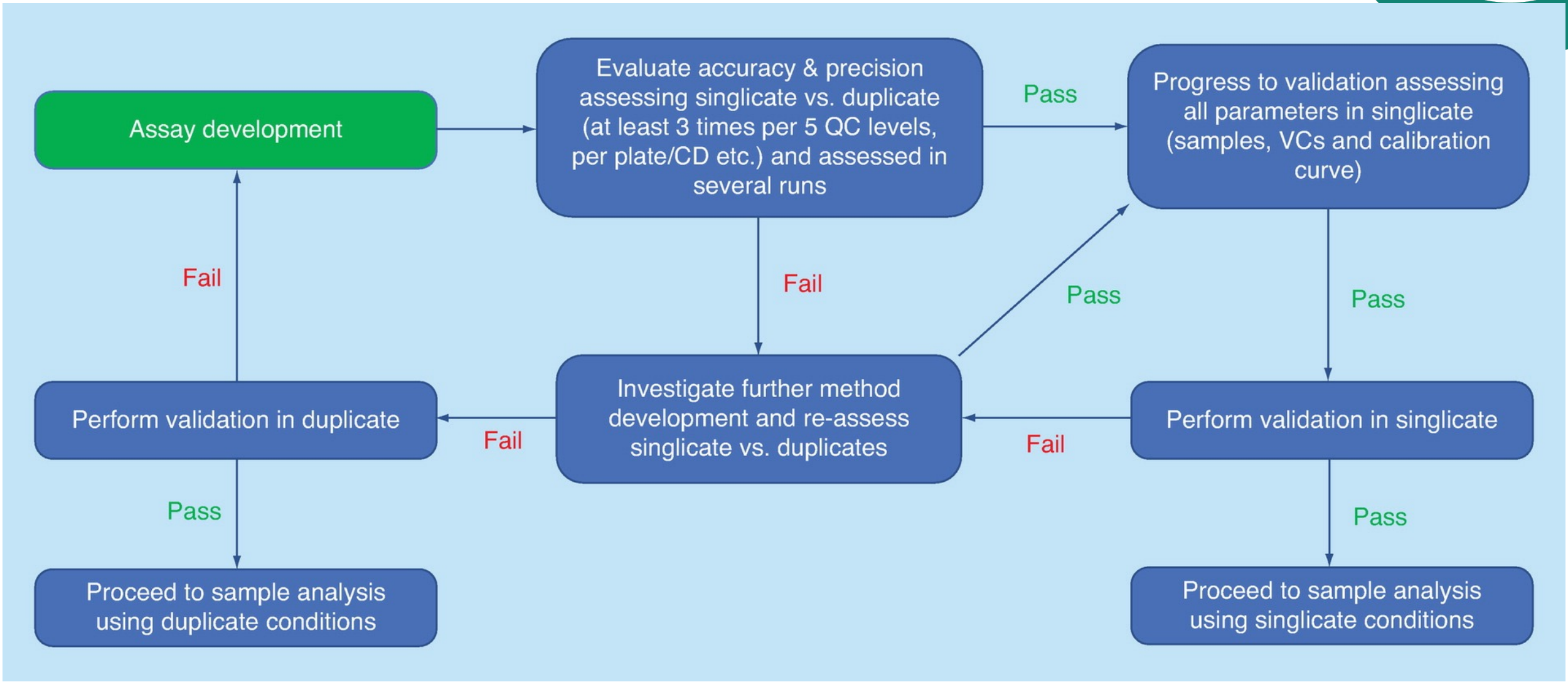
ICH M10 allows singlicate well analysis

Section 4.2: Validation

"When using LBA, study samples can be analysed using an assay format of 1 or more well(s) per sample. The assay format should be specified in the protocol, study plan or SOP. If method development and assay validation are performed using 1 or more well(s) per sample, then study sample analysis should also be performed using 1 or more well(s) per sample, respectively. If multiple wells per sample are used, the reportable sample concentration value should be determined either by calculating the mean of the responses from the replicate wells or by averaging the concentrations calculated from each response. Data evaluation should be performed on reportable concentration values."

ICH M10 guideline on bioanalytical method validation. Effective date: 23Jan2023

Example workflow for assessing singlicate analysis



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Experience in PK validation and bioanalysis studies

Validation (compound X)
- Original validation performed in duplicate
- Theoretical assessment performed on the original validation data set
- Validation performed in singlicate

Bioanalysis (compound X)
- Performed in duplicate

Validation (compound Y)
- Original validation performed in duplicate
- Theoretical assessment performed on the original validation data set

Bioanalysis (compound Y)
- Approximately 1500 samples analyzed in singlicate

Duplicate vs Singlicate A&P data in Validation

Validation (compound X)

		VAL A	VAL B	VAL C	VAL D	VAL E	OC A	OC B
Nominal	**(µg/mL)**	**5.00**	**10.0**	**20.0**	**50.0**	**66.0**	**100**	**500**
Duplicate measurement								
Mean	(µg/mL)	4.67	9.36	17.6	52.2	70.4	105	515
Overall bias	(%)	-6.6	-6.4	-12.2	4.3	6.7	5.4	2.9
Total CV	(%)	14.5	12.7	13.5	15.6	13.2	10.7	11.4
Total Error	(%)	21.0	19.1	25.7	20.0	19.9	16.1	14.4
Theoretical Singlicate measurement 1								
Mean	(µg/mL)	4.68	9.28	17.4	52.5	68.8	104	507
Overall bias	(%)	-6.4	-7.2	-13.2	4.9	4.3	4.2	1.3
Total CV	(%)	15.9	14.2	15.6	16.9	17.7	10.9	12.2
Total Error	(%)	22.2	21.4	28.8	21.8	22.0	15.0	13.5
Theoretical Singlicate measurement 2								
Mean	(µg/mL)	4.64	9.43	17.7	51.8	70.4	105	505
Overall bias	(%)	-7.3	-5.7	-11.3	3.6	6.7	4.9	1.0
Total CV	(%)	15.6	12.1	12.3	14.8	12.1	13.7	17.4
Total Error	(%)	22.9	17.8	23.6	18.4	18.8	18.6	18.4

P&A data on theoretical singlicate assessment not robust

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Perform validation in singlicate

Duplicate vs Singlicate A&P data in Validation

Validation (compound X)

		VAL A	VAL B	VAL C	VAL D	VAL E	OC A	OC B
Nominal	**(µg/mL)**	**5.00**	**10.0**	**20.0**	**50.0**	**66.0**	**100**	**500**
Duplicate measurement								
Mean	(µg/mL)	4.67	9.36	17.6	52.2	70.4	105	515
Overall bias	(%)	-6.6	-6.4	-12.2	4.3	6.7	5.4	2.9
Total CV	(%)	14.5	12.7	13.5	15.6	13.2	10.7	11.4
Total Error	(%)	21.0	19.1	25.7	20.0	19.9	16.1	14.4
Theoretical Singlicate measurement 1								
Mean	(µg/mL)	4.68	9.28	17.4	52.5	68.8	104	507
Overall bias	(%)	-6.4	-7.2	-13.2	4.9	4.3	4.2	1.3
Total CV	(%)	15.9	14.2	15.6	16.9	17.7	10.9	12.2
Total Error	(%)	22.2	21.4	28.8	21.8	22.0	15.0	13.5
Theoretical Singlicate measurement 2								
Mean	(µg/mL)	4.64	9.43	17.7	51.8	70.4	105	505
Overall bias	(%)	-7.3	-5.7	-11.3	3.6	6.7	4.9	1.0
Total CV	(%)	15.6	12.1	12.3	14.8	12.1	13.7	17.4
Total Error	(%)	22.9	17.8	23.6	18.4	18.8	18.6	18.4
Singlicate measurement								
Mean	(µg/mL)	4.92	9.97	18.9	48.4	65.6	99.8	487
Overall bias	(%)	-1.7	-0.3	-5.4	-3.2	-0.5	-0.2	-2.5
Total CV	(%)	19.6	15.0	18.2	20.1	19.2	18.8	21.1
Total Error	(%)	21.3	15.4	23.6	23.3	19.7	19.0	23.6

Singlicate analysis impacted the quality of the data

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Performed bioanalysis in duplicate

Duplicate vs Singlicate A&P data in Validation

Validation (compound Y)

		VAL A	VAL B	VAL C	VAL D	VAL E	OC A	OC B
Nominal	**(ng/mL)**	**5.00**	**15.0**	**150.0**	**3750.0**	**5000.0**	**150**	**37500**
Duplicate measurement								
Mean	(ng/mL)	4.88	14.7	145	3840	5120	154	38300
Overall bias	(%)	-2.5	-1.7	-3.6	2.5	2.4	2.8	2.2
Total CV	(%)	5.6	6.1	2.5	5.9	9.6	5.9	7.0
Total Error	(%)	8.1	7.8	6.1	8.4	12.1	8.7	9.1
Theoretical Singlicate measurement								
Mean	(ng/mL)	4.86	14.7	144	3870	5120	153	38300
Overall bias	(%)	-2.8	-2.3	-3.8	3.2	2.3	2.0	2.2
Total CV	(%)	5.4	6.3	2.5	5.7	9.7	6.4	7.7
Total Error	(%)	8.2	8.6	6.3	8.9	12.0	8.5	9.9

Minimal impact of singlicate analysis in validation

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Perform bioanalysis in singlicate

Other validation parameters (theoretical assessment) with singlicate analysis were all within acceptance criteria:

- Dilution linearity and Hook effect
- Selectivity in healthy and diseased matrix
- Freeze-thaw stability
- Bench-top stability
- Long term stability

Duplicate vs Singlicate data in Bioanalysis

Singlicate analysis

Bioanalysis study	Samples analyzed	#samples per plate	#plates	#Days	#ISR (%)
Study A	1576	78	20	20	95.2
Study B	167	78	3	3	NA
Study C	83	78	2	2	NA

Limitation: Only 1 plate per day was analyzed. Due to high number of dilutions and incubation time a total of 2 plates was not achievable.

Theoretical duplicate approach

Bioanalysis study	Samples analyzed	#samples per plate	#plates	#Days	#ISR (%)
Study A	1576	31	51	26	NC
Study B	167	31	6	3	NC
Study C	83	31	3	2	NC

- Singlicate analysis reduced number of days by 24% reduction (6 days, with 1 plate)
- Singlicate analysis increased the samples analyzed per day with 20% (78 vs 62)
- Singlicate approach reduced number of plates by 60% (31 plates less)
- Singlicate approach is cheaper and more sustainable (less plates, coating + detection / wash-/ block-/ read buffer)

Take home messages

- Singlicate analysis can impact data quality. Proceeding to bioanalysis in singlicate must always be data driven

- Singlicate analysis results in increased efficiency
  - run more samples in a shorter time frame
  - Reduced time to perform a study
- Singlicate analysis leads to improved sustainability
  - Less reagents, materials and buffers are used
  - Less bridging experiments required (often a critical experiment)

More to gain:

- Develop the assay fit for singlicate analysis (e.g. incubation times, sample dilution steps)
- Automated pipetting in singlicate (high throughput on multiple robots)
- Use 384 well plate for even higher throughput

Adjustments to be considered:

- Validation in Singlicate: A&P in 6-fold instead of 3-fold for better sample size?

Questions?

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