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Kinetic Modelling Shows Higher Accuracy in Shelf-life Predictions of Antibodies and Viral Vectors

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Introduction

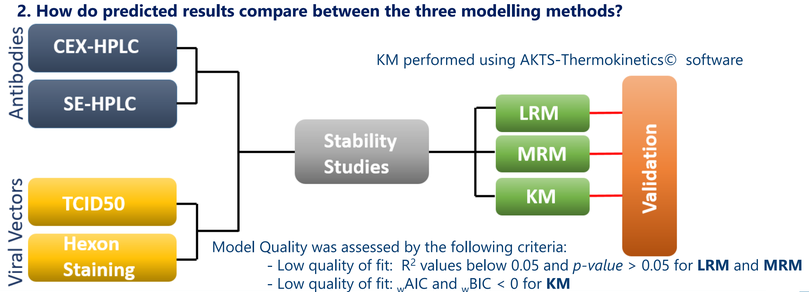
- Estimating shelf-life of drug products is crucial to ensure efficacy, purity and potency
- Shelf life is estimated from long term stability and accelerated aging studies
- The classical approach approved by ICH guidelines is to perform **linear regression** on stability attributes, on the condition that the degradation pattern is constant
- Previous research show that **alternative methods** to linear regression could better tackle the degradation of **antibodies [1, 2] and viral vectors [3, 4]** which exhibit complex degradation patterns composed of multiple steps and autocatalytic behaviour, leading to **superior stability predictions for selection candidates**

Here we compared the accuracy of **three predictive methods** on the stability of 2 antibodies and 2 viral vectors **after storage at 5°C and 25°C for 6 months** : linear (LRM), multiple regression (MRM) and kinetic modelling (KM).

Research Questions and Methods

1. How accurate are predictions using data up to 3m compared to measured data at 6m?

2. How do predicted results compare between the three modelling methods?



Model Quality was assessed by the following criteria:
- Low quality of fit: R^2 values below 0.05 and *p-value* > 0.05 for **LRM** and **MRM**
- Low quality of fit: $_wAIC$ and $_wBIC$ < 0 for **KM**

3. What is the minimum of time points necessary for accurate predictions?

Plus: Is there a modelling method working best for mAb vs. viral vectors?

Time points Used for Data Generation							
Temperature	0 weeks	1 week	2 week	1 month	2 months	3 months	6 months
5°C	X			X	X	X	X
25°C	X			X	X	X	X
40°C	X	X	X	X			

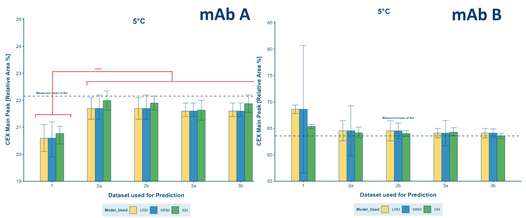
- Dataset 1: Using all data up to 1 month for three temperatures
- Dataset 2a: Using all data up to 2 months for three temperatures
- Dataset 2b: Using all data up to 2 months for 5°C, 25°C, and only t = 0, and t = 1 month data for 40°C
- Dataset 3a: Using all data up to 3 months for three temperatures
- Dataset 3b: Using all data up to 3 months for 5°C, 25°C, and only t = 0, and t = 1 month data for 40°C

Results

CEX-HPLC storage at 5°C

Modelling Method* (Dataset 3a)	Main Peak Area [%] Error [Measured - Predicted] mAb A	mAb B
LRM	0.56	**- 0.52**
MRM	0.56	**- 0.52**
KM	**0.52**	- 0.71

All models for both antibodies show less than 1% prediction error from measured stability at 6 months.



Any **dataset with 2 months or more data revealed to be significantly more accurate** in predicting 6 months stability **independently on the modelling methods used.**
(Two-way ANOVA significant main effect of Dataset ($p < 0.05$), confirmed by Post hoc Tukey's HSD Test, $p < 0.001$)

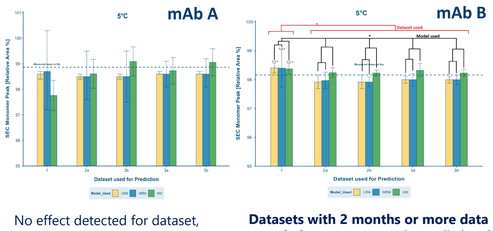
No effect detected for dataset, modelling method or their interactions. (Two-way ANOVA)

*All models showed high quality of fit.

SE-HPLC storage at 5°C

Modelling Method* (Dataset 3a)	Monomer Peak Area [%] Error [Measured - Predicted] mAb A	mAb B
LRM	**0.01**	**0.16**
MRM	**0.01**	**0.16**
KM	-0.11	**-0.16**

All models for both antibodies less than 1% prediction error from measured stability at 6 months.



No effect detected for dataset, modelling method or their interactions. (Two-way ANOVA)

*All models showed high quality of fit.

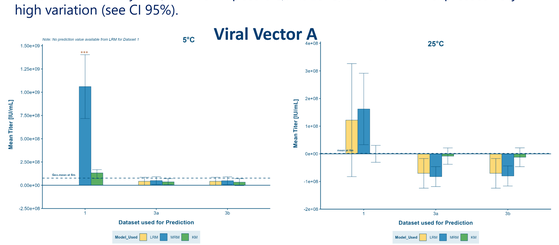
Datasets with 2 months or more data revealed same accuracy in predicting 6 months stability. **Kinetic Modelling was consistently more** accurate than LM and MRM.
(Two-way ANOVA significant main effect of Dataset and Model Used ($p < 0.05$) confirmed by Post hoc Tukey's HSD Test, $p < 0.022$ for Dataset, $p < 0.021$ for Model Used)

Hexon Staining storage at 5°C and 25°C

Modelling Method* (Dataset 3a)	Mean Titer [IU/ml] Error [Measured - Predicted] (Error %) 5°C	25°C
LRM	3.38E+07 (44.14%)	7.22E+07 (4204.56%)
MRM	**2.70E+07 (35.21%)**	8.41E+07 (4898.23%)
KM	4.35E+07 (56.81%)	**1.41E+07 (824.19%)**

KM revealed more reliable predictions of Mean Titer at 25°C.

* Most LRM models for stability at 5°C and 25°C showed low quality of fit and were removed from analyses (no ANOVA possible). At 25°C, LM and MRM expresses very high variation (see CI 95%).



For datasets with 3 months data, all methods were similarly accurate in predicting measured stability at 6 months.
(One-Sample t-test against mean Titer, $p < 0.038$ for Dataset with 1 month data and MRM)

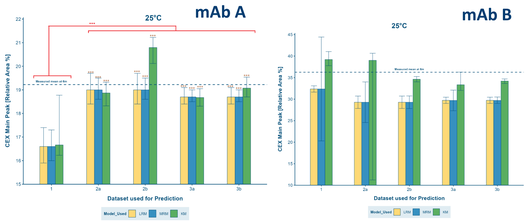
KM was the only method accurate enough to be compared to measured Titer, as seen by CI bars.
(One-Sample t-test against mean Titer, $p > 0.05$ for all methods and datasets)

CEX-HPLC storage at 25°C

Modelling Method* (Dataset 3a)	Main Peak Area [%] Error [Measured - Predicted] mAb A	mAb B
LRM	**0.52**	6.54
MRM	**0.52**	6.56
KM	0.55	**2.91**

mAb A: All models A showed less than 1% prediction error from measured stability at 6 months.

mAb B: KM showed the lowest prediction error from measured stability at 6 months.



Any **dataset with 2 months or more data revealed to be significantly more accurate** in predicting 6 months stability **independently on the modelling methods used.**
(Two-way ANOVA significant main effect of Dataset ($p < 0.05$), confirmed by Post hoc Tukey's HSD Test, $p < 0.001$)

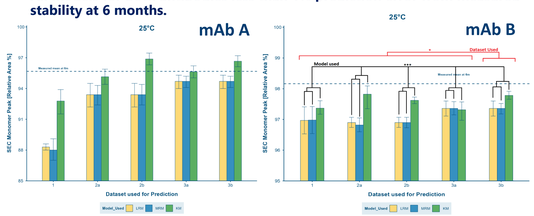
No effect detected of dataset, modelling method or their interactions. (Two-way ANOVA)

*All models showed high quality of fit.

SE-HPLC storage at 25°C

Modelling Method* (Dataset 3a)	Monomer Peak Area [%] Error [Measured - Predicted] mAb A	mAb B
LRM	0.89	**0.50**
MRM	0.89	**0.50**
KM	**0.59**	0.54

All models for both antibodies less than 1% prediction error from measured stability at 6 months.



Prediction accuracy at 6 months increases with increasing time points in the dataset and particularly with KM compared to LM and MRM.

(Two-way ANOVA significant main effect of Dataset, Model Used and their Interaction ($p < 0.05$) confirmed by Post hoc Tukey's HSD Test, $p < 0.001$ for Dataset, $p < 0.001$ for Model used and $p < 0.011$ for their Interaction)

Datasets with 3 months data showed to be more accurate in predicting 6 months data. KM revealed to be significantly more accurate in comparison to LM and MRM.

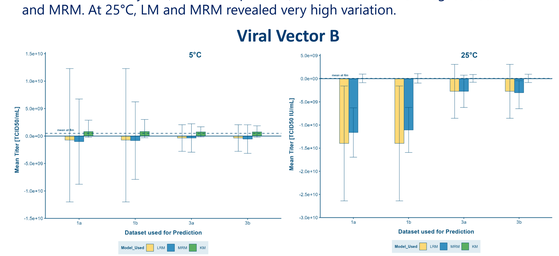
(Two-way ANOVA significant main effect of Dataset and Model Used ($p < 0.05$) confirmed by Post hoc Tukey's HSD Test, $p < 0.05$ for Dataset, $p < 0.001$ for Model Used)

TCID50 storage at 5°C and 25°C

Modelling Method (Dataset 3a) *	Mean Titer [TCID50/ml] Error [Measured - Predicted] (Error %) 5°C	25°C
LRM	8.60E+08 (170.61%)	2.80E+09 (3783.41%)
MRM	8.60E+08 (170.61%)	2.80E+09 (3783.41%)
KM	**-2.52E+08 (49.91%)**	**1.19E+07 (16.03%)**

KM revealed more reliable predictions of Mean Titer at 25°C.

* Most LRM models for stability at 5°C and25°C showed low quality of fit and were removed from analyses (no ANOVA possible). Predictions were on negative scale for LRM and MRM. At 25°C, LM and MRM revealed very high variation.



KM was the only method accurate enough to be compared to measured Titer, as seen by CI bars.
(One-Sample t-test against mean Titer, p > 0.05 for all methods and datasets)

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(One-Sample t-test against mean Titer, p > 0.05 for all methods and all datasets)

Discussion

Current ICH guidelines support the use of Linear Regression as golden standard to predict shelf life stability of drug products. In agreement with previous findings [1,2] kinetic modelling showed to be highly accurate in predicting degradation pathways of standard antibodies as measured by HPLC methods. Nevertheless, **linear and multiple regression models showed similar results in accuracy, supporting ICH suggestions**.

However, shortcomings of linear regression become evident when assays with high variability and complex degradation pathways are under analysis. **Kinetic modelling showed to be the only method able to handle higher temperature stresses [3, 4]** and provide meaningful insights in viral vectors degradation.

Summary

Stability prediction accuracy is **highly dependent on measurement** assays and drug substances. **Antibodies** measured with CEX and SEC showed **consistent results**, while **viral vectors** as measured by Hexon Staining and TCID50 presented a huge **modelling challenge**.

Antibody stability

- **All predictions at 5°C and 25°C for CEX and SEC** with training data up to 3 months showed **very high accuracy** compared to measured stability at 6 months
- All modelling methods were interchangeable in providing accurate results, but **Kinetic Modelling showed to be more accurate** with **increasing storage temperature**
- There is **no need to collect more than 2 months data** to predict 6 months stability for standard mAbs

Viral stability

- Predictions **at 5°C and 25°C for TCID50 and Hexon Staining** show degradation irregularities between training data up to 3 months and measured stability at 6 months which increased inaccuracy
- At 5°C storage, all modelling methods revealed inaccurate predictions. With increasing temperature, **at 25°C Kinetic Modelling showed consistently more accurate predictions** compared to measured stability at 6 months. Oppositely, linear regression consistently showed the lowest prediction outcome
- A **minimum of 3 months data** is strongly suggested to infer predictions for 6 months stability

References

[1] Kuzman, D., Bunc, M., Ravnik, M., Reiter, F., Žagar, L., & Bončina, M. (2021). Long-term stability predictions of therapeutic monoclonal antibodies in solution using Arrhenius-based kinetics. Scientific Reports, 11

[2] Evers, A., Clénet, D., & Pfeiffer-Marek, S. (2022). Long-Term Stability Prediction for Developability Assessment of Biopharmaceutics Using Advanced Kinetic Modeling. Pharmaceutics, 14(2), 375

[3] Clénet, D. (2018). Accurate prediction of vaccine stability under real storage conditions and during temperature excursions. European Journal of Pharmaceutics and Biopharmaceutics, 125, 76–84.

[4] Campa, C., Pronce, T., Paludi, M., Weusten, J., Conway, L., Savery, J., Richards, C., & Clénet, D. (2021). Use of Stability Modeling to Support Accelerated Vaccine Development and Supply. Vaccines, 9(10), 1114. https://doi.org/10.3390/vaccines9101114