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TITLE: A Two-Stage Bootstrap framework for long term stability modelling of biologics

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ABSTRACT: Predicting the long-term stability of biologics under intended storage conditions is a critical challenge in pharmaceutical development. Accelerated stability studies, conducted at elevated temperatures, are used as a base to forecast degradation behavior over extended timeframes. However, a fundamental assumption underlying these predictions; that a single kinetic equation and constant activation energy adequately describes degradation across all temperatures; may not be true for biological molecules, where multiple, mechanistically distinct degradation pathways can occur simultaneously and with different temperature dependencies.
The two-stage bootstrap method is different from using an Arrhenius approach with global kinetic modelling. Rather than one kinetic equation for all the temperatures, the two-stage bootstrap first estimates degradation rates independently at each temperature, before fitting the Arrhenius relationship across temperatures in a second stage. This separation better captures temperature-specific degradation behavior, and the bootstrapping provides more reliable uncertainty estimates, which is particularly advantageous when training data is sparse during early phases of biopharmaceutical development.
We compared the two-stage bootstrap and a global kinetic modelling method on SEC HMWS data collected at 5°C, 25°C, and 40°C over 3 months. Both models showed comparable predictive power at 6 months, validated against measured data. However significant difference arose between the two models at 2-year prediction, demonstrating the impact of model choice in long term stability predictions.
A more holistic approach involves a systematic comparison of activation energies from global kinetic models fitted independently across stability indicating attributes- including acidic variants and LMWS. Correlations between the activation energies provide insight into whether distinct temperature-dependent degradation mechanisms operate in parallel, and which pathways could dominate at the intended storage condition. We explore the multi activation energy profile comparison that can potentially serve as a rational basis for reflecting the true nature of degradation of the molecule.

Keywords: Two-Stage Bootstrap, Kinetic modelling, Long term stability prediction, Drug development