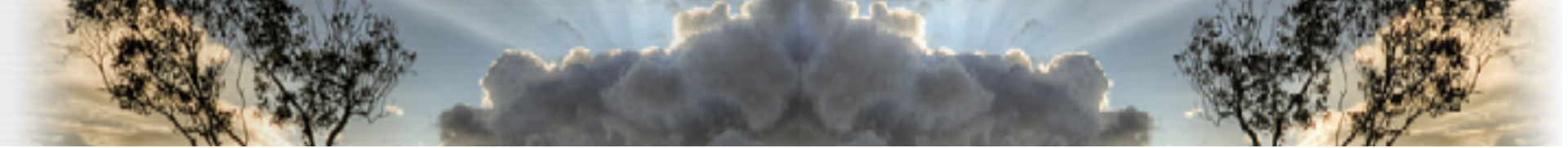

Benchtop and Freeze Thaw Stability

Timothy Sangster, on behalf of the EBF

Stability - ICH M10

- Stability evaluations should be carried out to ensure that every step taken during sample preparation, processing and analysis as well as the storage conditions used do not affect the concentration of the analyte.

- The storage and analytical conditions applied to the stability tests, such as the sample storage times and temperatures, sample matrix, anticoagulant and container materials, should reflect those used for the study samples. Reference to data published in the literature is not considered sufficient. Validation of storage periods should be performed on stability QCs that have been stored for a time that is equal to or longer than the study sample storage periods.

- Stability of the analyte in the studied matrix is evaluated using low and high concentration stability QCs. Aliquots of the low and high stability QCs are analysed at time zero and after the ICH M10 Guideline 14 applied storage conditions that are to be evaluated. A minimum of three stability QCs should be prepared and analysed per concentration level/storage condition/timepoint.

- The stability QCs are analysed against a calibration curve, obtained from freshly spiked calibration standards in a run with its corresponding freshly prepared QCs or QCs for which stability has been proven. The mean concentration at each QC level should be within ±15% of the nominal concentration. If the concentrations of the study samples are consistently higher than the ULOQ of the calibration range, the concentration of the high stability QC should be adjusted to reflect these higher concentrations. It is recognised that this may not be possible in nonclinical studies due to solubility limitations

- If multiple analytes are present in the study samples (e.g., studies with a fixed combination, or due to a specific drug regimen) the stability test of an analyte in matrix should be conducted with the matrix containing all of the analytes.

Ambiguity on Stability (may already be covered)

- ‘A minimum of three stability QCs should be prepared and analysed per concentration level/storage condition/timepoint’

- We feel the use of ‘prepared’ is ambiguous is this 3 replicate from 1 aliquot
- Or 3 aliquots and 1 analysis from each

- Suggestion would be 1 aliquot and 3 replicates from each

- Definitions –
  - Aliquot – separate tubes from a single preparation
  - Replicate - multiple sub-samples for analysis from single same tube

Stability - ICH M10

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- If multiple analytes are present in the study samples (e.g., studies with a fixed combination, or due to a specific drug regimen) the stability test of an analyte in matrix should be conducted with the matrix containing all of the analytes.

‘Freshly’ – ICH M10

- 317 The calibration curve should be prepared using freshly spiked calibration standards in at least one assessment. (Calibration curve and range)
- 350 The calibration curves for these assessments should be prepared using freshly spiked calibration standards in at least one run. (A&P)
- 396 The stability QCs are analysed against a calibration curve, obtained from freshly spiked calibration standards in a run with its corresponding freshly prepared QCs (Stability)
- 427 Stability QCs for freeze-thaw stability should be assessed using freshly prepared calibration standards and QCs (F/T Stability)
- 725 The calibration curve should preferably be prepared using freshly spiked calibration standards. (LBA Calibration curve and range)
- 799 The stability QCs are analysed against a calibration curve, obtained from freshly spiked calibration standards in a run with its corresponding freshly prepared QCs (LBA Stability)

Ambiguity on Stability

- '…from freshly spiked calibration standards in a run with its corresponding freshly prepared QCs…'
- What does this mean?
  - Prepared within 1 minute, 1 hour, 1 day?
  - Spiked – does this allow us to use spiking solutions or working solutions that are within stability (known or under investigation)?
  - Do we need have to go back to stocks prepared on the day?

- '…from freshly spiked calibration standards in a run with its corresponding freshly prepared QCs…'
  - How does this differ from freshly spiked?

Suggestion on Stability

- Freshly spiked or prepared are the same – suggest using prepared.

- Definition of fresh(ly) – prepared on the day of analysis and analysed within stability and the using intermediates which are within known stability (or to be proven stability).

Freeze-Thaw Matrix Stability ICH M10

- To assess the impact of repeatedly removing samples from frozen storage, the stability of the analyte should be assessed after multiple cycles of freezing and thawing. Low and high stability QCs should be thawed and analysed according to the same procedures as the study samples. Stability QCs should be kept frozen for at least 12 hours between the thawing cycles. Stability QCs for freeze-thaw stability should be assessed using freshly prepared calibration standards and QCs or QCs for which stability has been proven. The number of freeze-thaw cycles validated should equal or exceed that of the freeze-thaw cycles undergone by the study samples, but a minimum of three cycles should be conducted.

Bench Top (Short-Term) Matrix Stability ICH M10

- Bench top matrix stability experiments should be designed and conducted to cover the laboratory handling conditions for the study samples.
- Low and high stability QCs should be thawed in the same manner as the study samples and kept on the bench top at the same temperature and for at least the same duration as the study samples.
- The total time on the bench top should be concurrent; it is not acceptable to use additive exposure to bench top conditions (i.e., adding up time from each freeze-thaw evaluation is not acceptable).

Discussion Points on Bench Top Stability

- ‘kept on the bench top at the same temperature’ is this true when we put samples on ice do we monitor the temperature?
  - Suggest changing to …same conditions.

- Why is ‘it is not acceptable to use additive exposure to bench top conditions’?

Suggested comment to EMA/EWG

Final recommendation from this presentation, which combines the original recommendation enhanced with the discussions from the panel discussions during the meeting, are captured in the summary slide deck: Recommendations from the EBF Spring FW 2019

Acknowledgment and questions

- The EBF community for survey data and feedback
- Further questions to info@e-b-f.eu before 31 May