General Principles on Stability Testing – What is a Single Source

Presenter: Stuart McDougall,
on behalf of the EBF

Focus Workshop

(*In collaboration with the AAPS and JBF*)

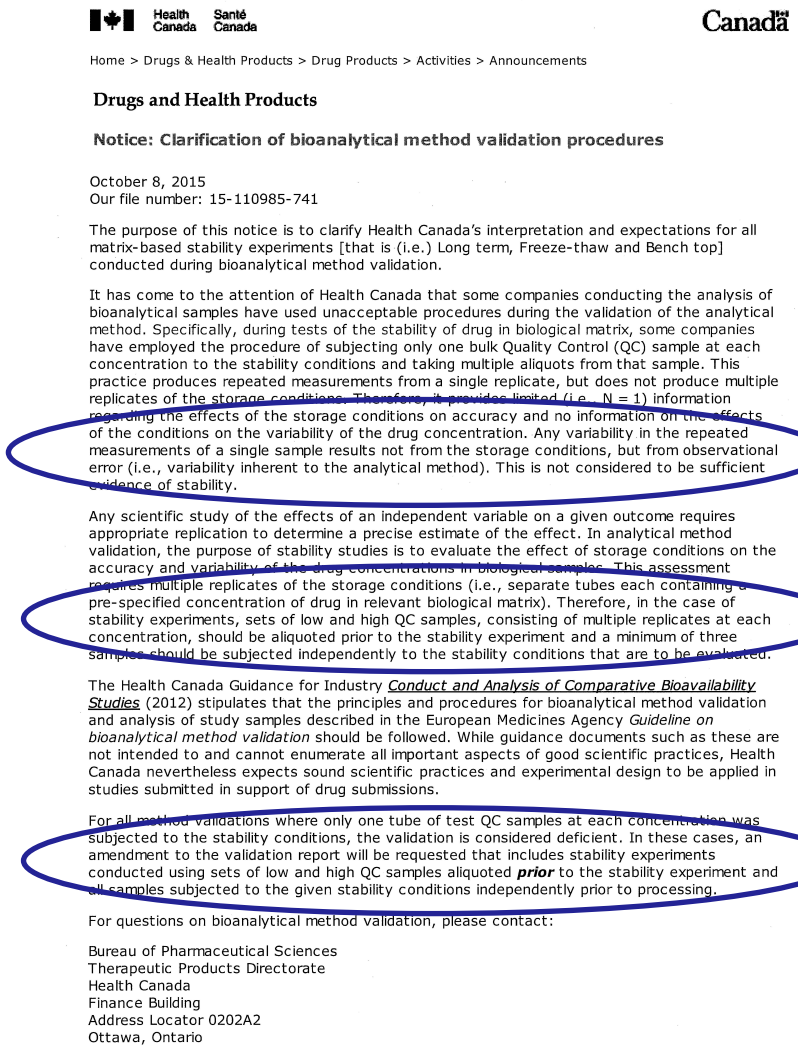
Industry input into ICH M10: Experimental data as the cornerstone for a science driven bioanalytical guideline

The Altis Grand Hotel Lisbon,
Portugal September 24-26, 2017

Samples, aliquots and replicates

- Ambiguity exists within current guidelines (and interpretation by industry)
  - FDA BMV (2001); 3 aliquots for FT, bench-top and long-term stability.
  - EMA BMV (2012); does not specify either aliquots or replicates for stability testing.
  - MHLW BMV (2013) Stability is evaluated by at least 3 replicates per concentration level.
  - CC-V workshop report (2014); 'Matrix related stability assessments should be conducted with at least three replicates at both the high and low concentrations within the validated range of the assay'.
  - GBC A6 Stability Team (AAPS 16(3) 2014, 392-399); 'A sufficient number of replicates should be performed to obtain a reliable average result for any stability assessment'.

HC Regulatory Position



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Drugs and Health Products

Notice: Clarification of bioanalytical method validation procedures

October 8, 2015
Our file number: 15-110985-741

The purpose of this notice is to clarify Health Canada's interpretation and expectations for all matrix-based stability experiments [that is (i.e.) Long term, Freeze-thaw and Bench top] conducted during bioanalytical method validation.

It has come to the attention of Health Canada that some companies conducting the analysis of bioanalytical samples have used unacceptable procedures during the validation of the analytical method. Specifically, during tests of the stability of drug in biological matrix, some companies have employed the procedure of subjecting only one bulk Quality Control (QC) sample at each concentration to the stability conditions and taking multiple aliquots from that sample. This practice produces repeated measurements from a single replicate, but does not produce multiple replicates of the storage conditions. Therefore, it provides limited (i.e., N = 1) information regarding the effects of the storage conditions on accuracy and no information on the effects of the conditions on the variability of the drug concentration. Any variability in the repeated measurements of a single sample results not from the storage conditions, but from observational error (i.e., variability inherent to the analytical method). This is not considered to be sufficient evidence of stability.

Any scientific study of the effects of an independent variable on a given outcome requires appropriate replication to determine a precise estimate of the effect. In analytical method validation, the purpose of stability studies is to evaluate the effect of storage conditions on the accuracy and variability of the drug concentrations in biological samples. This assessment requires multiple replicates of the storage conditions (i.e., separate tubes each containing a pre-specified concentration of drug in relevant biological matrix). Therefore, in the case of stability experiments, sets of low and high QC samples, consisting of multiple replicates at each concentration, should be aliquoted prior to the stability experiment and a minimum of three samples should be subjected independently to the stability conditions that are to be evaluated.

The Health Canada Guidance for Industry *Conduct and Analysis of Comparative Bioavailability Studies* (2012) stipulates that the principles and procedures for bioanalytical method validation and analysis of study samples described in the European Medicines Agency *Guideline on bioanalytical method validation* should be followed. While guidance documents such as these are not intended to and cannot enumerate all important aspects of good scientific practices, Health Canada nevertheless expects sound scientific practices and experimental design to be applied in studies submitted in support of drug submissions.

For all method validations where only one tube of test QC samples at each concentration was subjected to the stability conditions, the validation is considered deficient. In these cases, an amendment to the validation report will be requested that includes stability experiments conducted using sets of low and high QC samples aliquoted ***prior*** to the stability experiment and all samples subjected to the given stability conditions independently prior to processing.

For questions on bioanalytical method validation, please contact:

Bureau of Pharmaceutical Sciences
Therapeutic Products Directorate
Health Canada
Finance Building
Address Locator 0202A2
Ottawa, Ontario

- Long-term, Free/thaw & bench-top stability.
- Analysis of <u>replicates</u> from single sample is <u>not acceptable.</u>
- Must be done by analysis of at least 3 <u>separate samples.</u>
- HC consider validation deficient and retrospective.

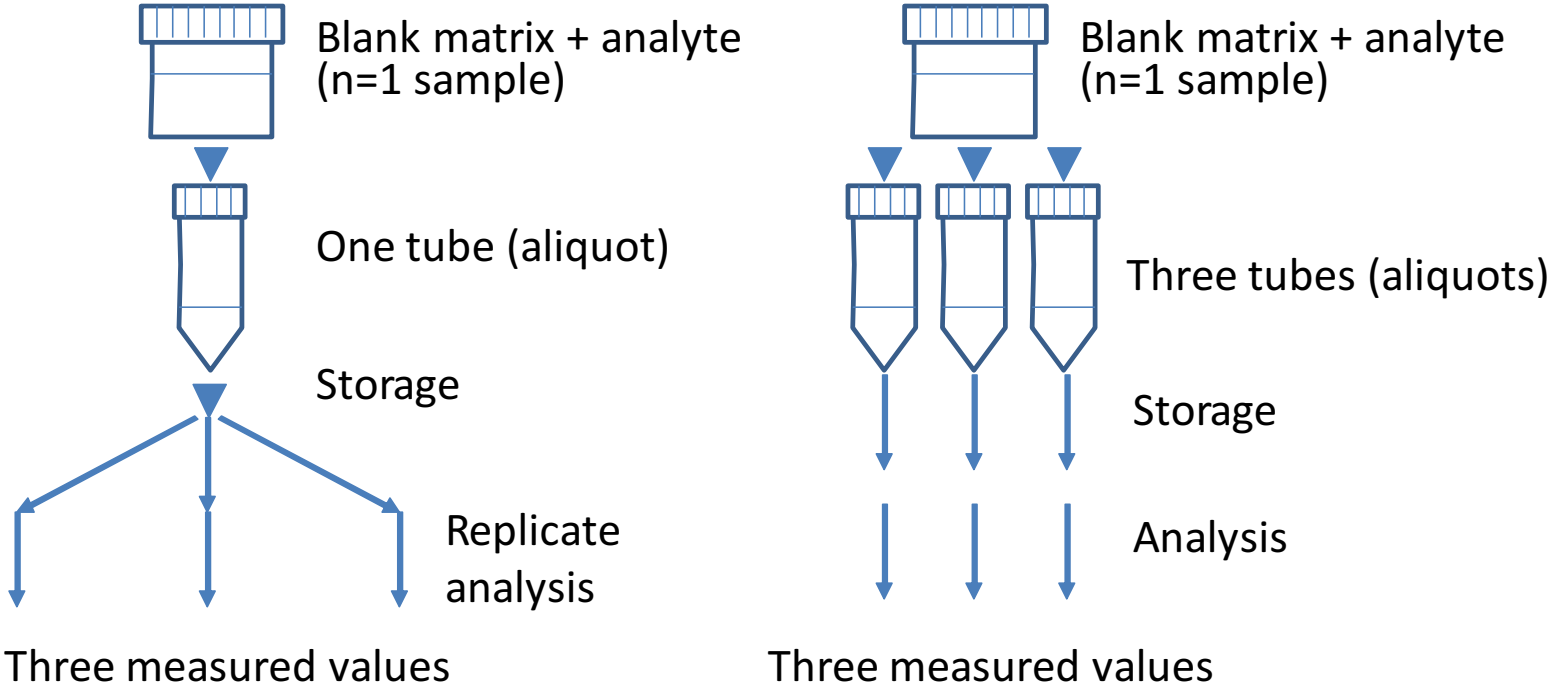
Definitions

- Sample – a small quantity (of something) from which the general quality (of the whole) may be inferred
- Aliquot - In analytical chemistry, the term aliquot is generally used to define any representative portion of the sample.
- Replicate Analysis or Measurement - The repeated analysis or measurement of the variable of interest performed as identically as possible.
- Replicate analyses are used to assess analytical or measurement variance and reduce uncertainty of measurement (UM).

Samples, aliquots and replicates

- Experimental design is dependent on investigation
  - Does drug concentration change when stored frozen in human plasma? (hypothesis; it changes, null-hypothesis; it does not change).
- What are the variables?
  - Dependent variable (what we measure); drug concentration.
  - Independent variables; temperature (x ± y°C) and time (x ± y hours).
  - Other experimental variables; analytical method, matrix (including anticoagulant), tube type, etc. (controlled).
- What else?
  - There is an uncertainty of measurement (UM) associated with the analysis (≤ +/-15% for SMOL and ≤ +/-20% for LBA).

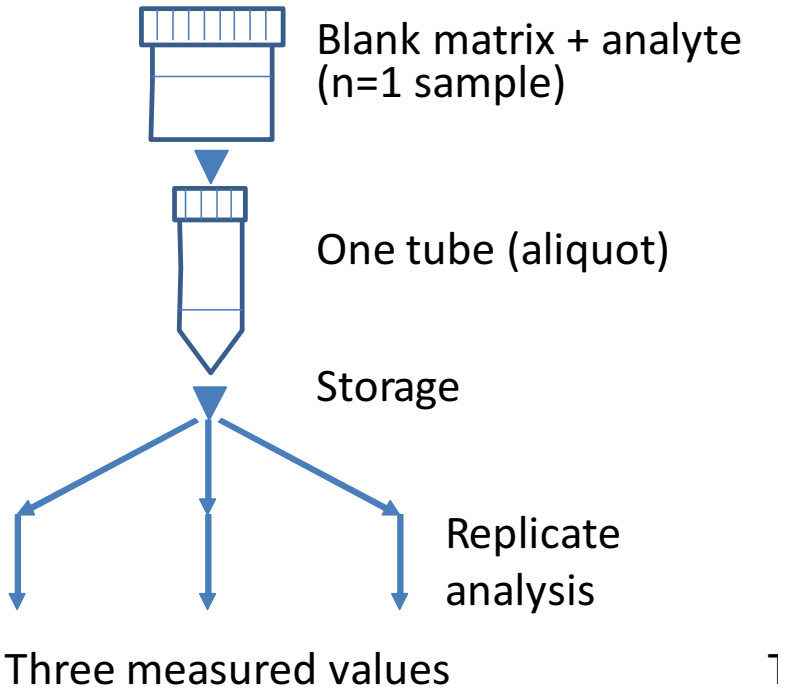
Samples, aliquots and replicates



- Replicate analysis will reduce uncertainty of measurement but <u>will not</u> increase the number of independent measurements.

Recommendation

- One ‘sample’ prepared at each drug level (i.e. QCL & QCH).
- Single aliquot subjected to test condition (temp/time)
- Replicates (≥ 3) analysed from aliquot.
- Fresh calibration (for long-term frozen stability testing) and QC control.
- Mean bias within ± 15% (for chromatographic) and ± 20% (for LBA) of nominal drug concentration.
- Multiple ‘tubes’ will increase cost, potentially require more matrix (unethical) and will not improve data quality



Summary

- Stability tests should support study samples (collection, transport, storage and analysis)
  - Long-term frozen
  - Matrix bench-top
  - Freeze/thaw
- Design testing such that dependent variable, independent variables and experimental variables are defined and controlled
- A single sample (with replicate analysis) is sufficient for each stability assessment at each concentration.

'It is not enough to do your best; you must know what to do, and then do your best' - W. Edwards Deming

EBF

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Contact: info@europeanbioanalysisforum.eu