Workshop

Towards harmonised implementation of the ICH M10 Guideline

3. Chromatography

Stuart McDougall and Iain Love,
on behalf of the EBF

15 November 2022, Barcelona

Flow of the session (3. Chromatography)

- What is explicitly stated in the Guideline
- Comments and questions raised by us
- Discussion and our consensus understanding
- AAPS perspective *(not uploaded on EBF website)*
- Action and follow-up

ICH guideline M10 on bioanalytical method validation

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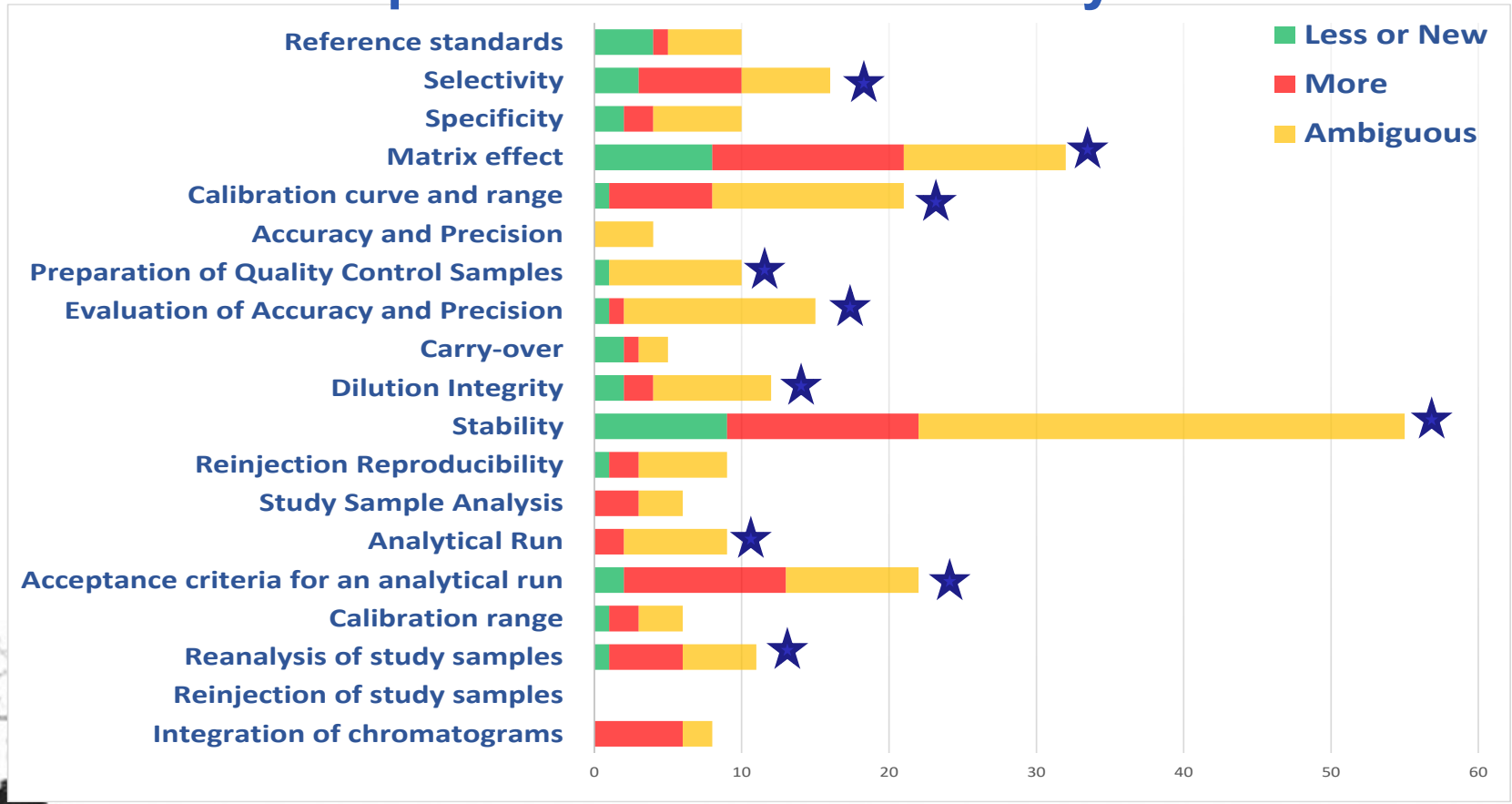
Themes/questions discussed today



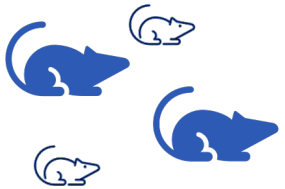
A reminder……

This guideline intends to facilitate development of drugs in accordance with the principles of 3Rs (Reduce, Refine, Replace) for animal studies, where valid.

and

In the cases of rare matrices, use of a surrogate matrix for dilution may be acceptable. It should be demonstrated that this does not affect precision and accuracy.

Rare (of a thing); not found in large number (or amount) and so of interest or value



Flow of the session (3. Chromatography)

- Section 3.1 (Reference standards)
- Section 3.2 (Validation)

ICH guideline M10 on bioanalytical method validation

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3.1 Reference Standards

3.1. Reference standards

During method validation and the analysis of study samples, a blank biological matrix is spiked with the analyte(s) of interest using solutions of reference standard(s) to prepare calibration standards and QCs. Calibration standards and QCs should be prepared from separate stock solutions. However, calibration standards and QCs may be prepared from the same stock solution provided the accurate preparation and stability of the stock solution should have been verified.

A suitable internal standard (IS) should be added to all calibration standards, QCs and study samples during sample processing. The absence of an IS should be justified.

It is important that the reference standard is well characterised and the quality (e.g., purity, identity) of the reference standard and the suitability of the IS is ensured, as the quality will affect the outcome of the analysis and, therefore, the study data. The reference standard used during validation and study sample analysis should be obtained from an authentic and traceable source. The reference standard should be identical to the analyte. If this is not possible, an established form (e.g., salt or hydrate) of known quality should be used.

Suitable reference standards include compendial standards, commercially available standards or sufficiently characterised standards prepared in-house or by an external organisation. A certificate of analysis (CoA) or an equivalent alternative is required to ensure quality and to provide information on the purity, storage conditions, retest/expiration date and batch number of the reference standard.

A CoA is not required for the IS as long as the suitability for use is demonstrated, e.g., a lack of analytical interference is shown for the substance itself or any impurities thereof.

When MS detection is used, the use of the stable isotope-labelled analyte as the IS is recommended whenever possible. However, it is essential that the labelled standard is of high isotope purity and that no isotope exchange reaction occurs. The presence of unlabelled analyte should be checked and if unlabelled analyte is detected, the potential influence should be evaluated during method validation.

Stock and working solutions should only be prepared from reference standards that are within the stability period as documented in the CoA (either expiration date or the retest date).

- Separate primary stocks (or same if verified ± 5%)
- Analyte (CoA or equivalent; purity, identity, stability)
- Internal standard (suitability for use)

ICH-M10 FAQ (22 May 2022). Is it acceptable to demonstrate the absence of analytical interference of the IS itself, any impurities or its isotopic stability based on the results of the zero sample?

ICH-M10 FAQ (22 May 2022). Yes, this is acceptable for both method validation and study sample analysis

ICH-M10 FAQ (22 May 2022). How is the accurate preparation of the stock solution verified?

ICH-M10 FAQ (22 May 2022). By comparing two independently prepared stock solutions and demonstrating that the difference of measured response is within 5% difference = ([stock solution 1 – stock solution 2]) / (mean value) x 100.

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Suitable… sufficien… …te of analysis… …ion on the purit… …d.

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When MS… …nded whenev… …d that no isoto… …if unlabell… …tion.

Stock a… …e stability…

A. A CoA is not required for the IS. Should we interpret that a Certificate of Identity is required but not a CoA?

A. *essential that the labelled standard is of high isotope purity and that no isotope exchange reaction occurs. The presence of unlabelled analyte should be checked* - must this be clearly tested in the scope of a validation?

L. Single stock for Cal/QC preparation is not currently in play within my company. It is anticipated this will help reduce the number of repeat analyses

ICH-M10 FAQ (22 May 2022). How is the accurate preparation of the stock solution verified?

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A. retest/expiry dates currently causing local tension as described by OECD19. MHRA asking for greater burdens of evidence as to the veracity of expiry dates.

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3.2.1 Selectivity ★

3.2. Validation

3.2.1. Selectivity

Selectivity is the ability of an analytical method to differentiate and measure the analyte in the presence of potential interfering substances in the blank biological matrix.

Selectivity should be evaluated using blank samples (matrix samples processed without addition of an analyte or IS) obtained from at least 6 individual sources/lots (non-haemolysed and non-lipaemic). Use of fewer sources may be acceptable in the case of rare matrices. Selectivity for the IS should also be evaluated.

The evaluation of selectivity should demonstrate that no significant response attributable to interfering components is observed at the retention time(s) of the analyte or the IS in the blank samples. Responses attributable to interfering components should not be more than 20% of the analyte response at the LLOQ and not more than 5% of the IS response in the LLOQ sample for each matrix.

For the investigation of selectivity in lipaemic matrices at least one source of matrix should be used. To be scientifically meaningful, the matrix used for these tests should be representative as much as possible of the expected study samples. A naturally lipaemic matrix with abnormally high levels of triglycerides should be obtained from donors. Although it is recommended to use lipaemic matrix from donors, if this is difficult to obtain, matrix can be spiked with triglycerides even though it may not be representative of study samples. However, if the drug impacts lipid metabolism or if the intended patient population is hyperlipidaemic, the use of spiked samples is discouraged. This evaluation is not necessary for nonclinical studies unless the drug impacts lipid metabolism or is administered in a particular animal strain that is hyperlipidaemic.

For the investigation of selectivity in haemolysed matrices at least one source of matrix should be used. Haemolysed matrices should be obtained by spiking matrix with haemolysed whole blood (at least 2% V/V) to generate a visibly detectable haemolysed sample.

- Analyte vs potential endogenous interferences

- 6 lots of blank matrix
- 1 lot Lipaemic (human*)
- 1 lot haemolysed (2% v/v)

- ≤ 20% of response of LLOQ at RT
- ≤ 5% response of IS at RT

ICH-M10 Section 2.2.1. Matrix differences within species (e.g. age, ethnicity, gender) are generally not considered different when validating a method

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Rare = 3Rs?

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ICH-M10 Section 2.2.1. Matrix differences within species (e.g. age, ethnicity, gender) are generally not considered different when validating a method

3.2.1 Selectivity ★

L. Good that lipaemic not required for non clinical

A. Can pooled lipemic and haemolysed plasma be used as one source?

ogenous

presence of potential interfering substances in the blank biological matrix.

L. allowance for use of fewer lots of rare matrices

M. Sourcing naturally lipaemic matrix maybe problematic

Plot Lipaemic (human*)

M. Specific assessments in lipemic and hemolyzed in addition to 6 normal lots

M. More samples if LLOQ samples for each matrix have to be prepared

A. Has selectivity failed when haemolytic or lipemic matrix has failed?

- ≤ 20% of response at LLOQ at RT
- ≤ 5% response of IS at RT

A. Is an investigation on failed selectivity of haemolytic/lipemic matrix sufficient or should assay be revised?

considered different when validating a method

A. Any advice on how to prepare 2% v/v haemolysed plasma?

Ref EBF; Bioanalysis (2014) 6(23) 3113-3120)

Discussion - Actions – proposal (Selectivity)

- Do we understand design?
- Would you support using fewer lots for ‘rare matrices’ (3Rs)?
- What to do if selectivity fails?

- Any other comments or questions?

3.2.2 Specificity

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Specificity is the ability of a bioanalytical method to detect and differentiate the analyte from other substances, including its related substances (e.g., substances that are structurally similar to the analyte, metabolites, isomers, impurities, degradation products formed during sample preparation, or concomitant medications that are expected to be used in the treatment of patients with the intended indication).

If the presence of related substances is anticipated in the biological matrix of interest, the impact of such substances should be evaluated during method validation, or alternatively, in the pre-dose study samples. In the case of LC-MS based methods, to assess the impact of such substances, the evaluation may include comparing the molecular weight of a potential interfering related substance with the analyte and chromatographic separation of the related substance from the analyte.

Responses detected and attributable to interfering components should not be more than 20% of the analyte response at the LLOQ and not more than 5% of the IS response in the LLOQ sample.

The possibility of back-conversion of a metabolite into the parent analyte during the successive steps of the analysis (including extraction procedures or in the MS source) should also be evaluated when relevant (e.g., potentially unstable metabolites such as ester analytes to ester/acidic metabolites, unstable N-oxides or glucuronide metabolites, lactone-ring structures). It is acknowledged that this evaluation will not be possible in the early stages of drug development of a new chemical entity when the metabolism is not yet evaluated. However, it is expected that this issue should be investigated, and partial validation performed if needed. The extent of back-conversion, if any, should be established and the impact on the study results should be discussed in the Bioanalytical Report.

- Analyte vs other potential exogenous interferences
- Not more than 20% of analyte response at LLOQ
- Not more than 5% of IS response
- In-silico for LC-MS; based on physicochemical properties (e.g. mass, pKa, Log P, etc)

ICH-M10 FAQ (22 May 2022). Can the physicochemical properties of the related substances be used to justify that the related substances do not co-elute or interfere with the analyte measurement during mass spectrometry analysis?

ICH-M10 FAQ (22 May 2022).
Yes, but if co-elution of the related substance and the analyte is not excluded, additional investigations are needed to demonstrate chromatographic separation (e.g. for isomers). If the analyte and the related substance co-elute, matrix effect (ion suppression/enhancement) and back conversion should be evaluated

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3.2.2 Specificity

- Analyte vs other potential exogenous

L. For LC/MS assay lack of interference from other drugs can be evaluated with a paper based approach - initiated at risk already with draft guidance and with support from EBF data

L. M10 permits specificity assessment based on mol. Weight. Actual assessment not required

3.2.2.

Specifici… …om other substanc… …to the analyte, …paration, concomi… …e intended indicatio…

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Ref EBF; Bioanalysis (2016) 8(19) 2065-2070

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M. Back-conversion of metabolite to parent needs to be assessed.

A. Does evaluation mean paper exercise? When is this not appropriate? Fixed dose combination therapies? Since these must be covered for stability assume the T0 assessment can be used for specificity. Is it only necessary to cover other SM analytes for SM methods or also biologics? If two analytes are very structurally similar then assume that justification can be used that they will not co-elute and will not interfere and no experimental work required? FAQ implies experimentally needs to be confirmed

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A. The paragraph on back-conversion is also very "open" with lot of room for interpretation

3.2.3 Matrix Effect★

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A matrix effect is defined as an alteration of the analyte response due to interfering and often unidentified component(s) in the sample matrix. During method validation the matrix effect between different independent sources/lots should be evaluated.

The matrix effect should be evaluated by analysing at least 3 replicates of low and high QCs, each prepared using matrix from at least 6 different sources/lots. For each individual matrix sources/lots evaluated, the accuracy should be within ±15% of the nominal concentration and the precision (per cent coefficient of variation (%CV)) should not be greater than 15%. Use of fewer sources/lots may be acceptable in the case of rare matrices.

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ICH-M10 Section 2.2.1. Matrix differences within species (e.g. age, ethnicity, gender) are generally not considered different when validating a method

- Potential impact vs analyte response
- 6 Lots
- 2 Levels (LOW and HIGH QC)
- 3 replicates (6 x 2 x 3 = 36)
- Accuracy @ ≤ ± 15% (per lot)
- Precision @ ≤ ± 15% (per lot)
- \+ in ‘special population’ (e.g renal or hepatic impaired)
- Include haemolysed & lipaemic if **likely** to occur

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Rare = 3Rs?

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3.2.3 Matrix Effect★

A. Does this mean that for haemolysed sample and lipaemic sample also 2 x 3 x 1 each are expected with the same criteria?

L. Determination of A&P in 6 matrices replaces matrix factor. Test is probably easier to be performed.

A. This leads to 3 replicates x 6 lots x 2 levels = 36 samples for the matrix effect test. Is this correct?

A. it is unclear what "relevant patient populations" are and when haemolytic and lipidemic matrix is in scope for matrix effect

L. Less work compared to the assessment and evaluation of matrix factors, but probably a higher risk of failure because of the criteria per lot of matrix

➢ 6 Lots

A. I read this as spiking, preparation and analysis of 3 x 6 spiked samples at low and high QC level, so 36 samples in total. The spiking should now to be done BEFORE sample preparation? What about the influence of recovery on this approach?

3 = 36)

The matrix effect should also be evaluated in relevant patient populations or special po

L. Matrix factor/IS normalised matrix factor reporting reduced

A. What about determination of matrix factor?

% (per lot)

M. Increase in the amount of preparation required. Now need to make QCs LOW and HIGH in different lots/sources which can extend analysis over one plate of samples.

A. What to do if 1 sample is not evaluable? If you have 3 replicates and you loose one you can´t calculate CV.

A. Which are the relevant patient populations? Same as the ones listed in 4.2.2?

generally not considered differ

M. 3 replicates in 6 sources at L and H

A. Why 3 replicates per sample?

M. Different from procedure in place at the moment. Implementation impact: i) more work; ii) more quantity of matrix to be used; iii) increase possibility of preparation error

Discussion - Actions – proposal (Matrix Effect)

- Do we understand design?
- Would you support using fewer lots/batches for ‘rare matrices’ (3Rs)?
- Do you agree that you are more likely to receive haemolysed samples rather than lipemic samples and validate accordingly?

- Any other comments or questions?

3.2.4 Calibration Curve and Range★

3.2.4. Calibration curve and range

The calibration curve demonstrates the relationship between the nominal analyte concentration and the response of the analytical platform to the analyte. Calibration standards, prepared by spiking matrix with a known quantity of analyte(s), span the calibration range and comprise the calibration curve. Calibration standards should be prepared in the same biological matrix as the study samples. The calibration range is defined by the LLOQ, which is the lowest calibration standard, and the ULOQ, which is the highest calibration standard. There should be one calibration curve for each analyte studied during method validation and for each analytical run.

A calibration curve should be generated with a blank sample, a zero sample (blank sample spiked with IS), and at least 6 concentration levels of calibration standards, including the LLOQ and the ULOQ.

A simple regression model that adequately describes the concentration-response relationship should be used. The selection of the regression model should be directed by written procedures. The regression model, weighting scheme and transformation should be determined during the method validation. Blank and zero samples should not be included in the determination of the regression equation for the calibration curve. Each calibration standard may be analysed in replicate, in which case data from all acceptable replicates should be used in the regression analysis.

The calibration curve parameters should be reported (e.g., slope and intercept in the case of a linear model). The back-calculated concentrations of the calibration standards should be presented together with the calculated mean accuracy and precision values. All acceptable curves obtained during validation, based on a minimum of 3 independent runs over several days, should be reported. The accuracy of the back-calculated concentrations of each calibration standard should be within ±20% of the nominal concentration at the LLOQ and within ±15% at all the other levels. At least 75% of the calibration standards with a minimum of 6 calibration standard levels should meet the above criteria.

In the case that replicates are used, the criteria (within ±15% or ±20% for LLOQ) should also be fulfilled for at least 50% of the calibration standards tested per concentration level. In the case that a calibration standard does not comply with these criteria, this calibration standard sample should be rejected, and the calibration curve without this calibration standard should be re-evaluated, including regression analysis. For accuracy and precision runs, if all replicates of the LLOQ or the ULOQ calibration standard in a run are rejected, then the run should be rejected, the possible source of the failure should be determined and the method revised, if necessary. If the next validation run also fails, then the method should be revised before restarting validation.

The calibration curve should be prepared using freshly spiked calibration standards in at least one assessment. Subsequently, frozen calibration standards can be used within their defined period of stability.

- A @ ≤ ±15% (± 20% at LLOQ)
- ≥ 75% of all standards
- ≥ 6 levels
- ≥ 50% / level for replicates

- ‘Simple’ regression model (SOP)

- ‘Freshly’ prepared for at least one run.

3.2.4 Calibration Curve and Range★

3.2.4. Calibration curve and range

The calibration curve demonstrates the relationship between the nominal analyte concentration and the response of the analytical platform to the analyte. Calibration standards, prepared by spiking matrix with a known quantity of analyte(s), span the calibration range and comprise the calibration curve. Calibration standards should be prepared in the same biological matrix as the study samples. The calibration range is defined by the LLOQ, which is the lowest calibration standard, and the ULOQ, which is the highest calibration standard. There should be one calibration curve for each analyte studied during method validation and for each analytical run.

A calibration curve should be generated with a blank sample, a zero sample (blank sample spiked with IS), and at least 6 concentration levels of calibration standards, including the LLOQ and the ULOQ.

A simple regression model that adequately describes the concentration-response relationship should be used. The selection of the regression model should be directed by written procedures. The regression model, weighting scheme and transformation should be determined during the method validation. Blank and zero samples should not be included in the determination of the regression equation for the calibration curve. Each calibration standard may be analysed in replicate, in which case data from all acceptable replicates should be used in the regression analysis.

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Linear < Quad?
$x < 1/x < 1x^2$?

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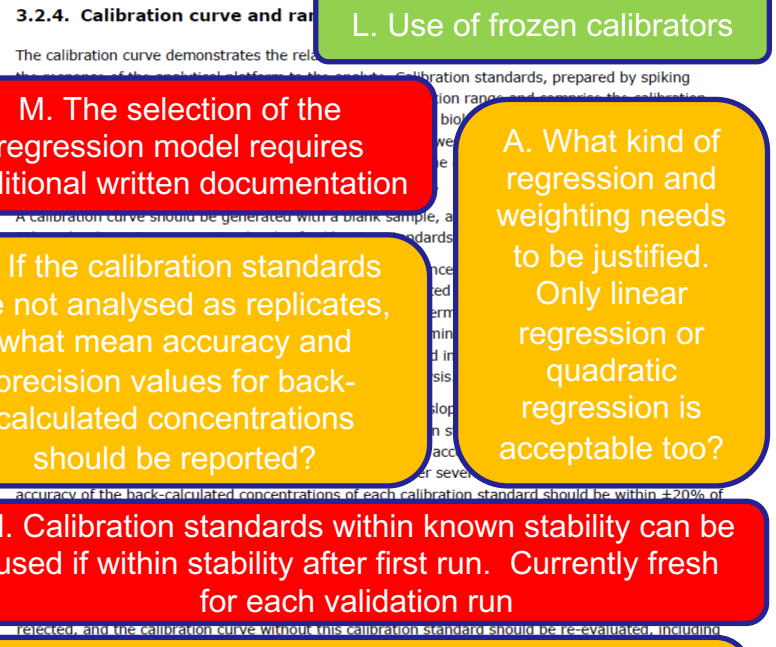
3.2.4 Calibration Curve and Range★


3.2.4. Calibration curve and ran

The calibration curve demonstrates the rela

Calibration standards, prepared by spiking

A calibration curve should be generated with a blank sample, a

accuracy of the back-calculated concentrations of each calibration standard should be within ±20% of

rejected, and the calibration curve without this calibration standard should be re-evaluated, including

L. Use of frozen calibrators

M. The selection of the regression model requires additional written documentation

A. What kind of regression and weighting needs to be justified. Only linear regression or quadratic regression is acceptable too?

A. If the calibration standards are not analysed as replicates, what mean accuracy and precision values for back-calculated concentrations should be reported?

M. Calibration standards within known stability can be used if within stability after first run. Currently fresh for each validation run

A. Not clear if we should demonstrate and generate raw data on how we have chosen the simplest regression model or if it is fine to use directly a simple model with a preset weighting (e.g. linear regression with 1/x2 weighting)?

A. CAL samples could be used frozen after the first run. Not in accordance with the FDA?

A. Does rejecting a complete validation run if all ULOQ or LLOQ replicates are rejected only apply to calibration with replicates??

A. if both replicates at one level failed, is the run invalid or can just the STD be removed as applied today?

A. "Each calibration standard may be analysed in replicate" means that the scientist can decide about number of replicates for each level, blank and zero individually (e.g. 2 x blank, 1 x zero, 1 x LLOQ, 2x (3x LLOQ level), 2x ULOQ and all others 1x). Was this really the intention and how do EBF members apply

A. Is it enough to describe in the SOP that always $1/x^2$ is used? Or should an evaluation be performed for each method after the A+P runs?

A. What if only freshly prepared calibration standards are used, for all assessments?

Discussion - Actions – proposal(Calibration Curve and Range)

- Do we understand design?
- ‘How do we interpret ‘A simple regression model that adequately describes the concentration-response relationship should be used. The selection of the regression model should be directed by written procedures’? Would it be sufficient to state linear < quadratic and $1/x^2$ in SOP?

- Any other comments or questions?

3.2.5 Accuracy and Precision
3.2.5.1 Preparation of Quality Control Samples ★

3.2.5. Accuracy and precision

3.2.5.1. Preparation of quality control samples

The QCs are intended to mimic study samples and should be prepared by spiking matrix with a known quantity of analyte, storing them under the conditions anticipated for study samples and analysing them to assess the validity of the analytical method.

Calibration standards and the QCs should be prepared from separate stock solutions in order to avoid biased estimations which are not related to the analytical performance of the method. If calibration standards and the QCs may be prepared from the same stock solution, the accuracy and stability of the stock solution should be verified. A single source of blank matrix may be used, which should be free of interference or matrix effects, as described in Section 3.2.3.

During method validation the QCs for accuracy and precision runs should be prepared at a minimum of 4 concentration levels within the calibration curve range: the LLOQ, within three times of the LLOQ (low QC), around 30 - 50% of the calibration curve range (medium QC) and at least 75% of the ULOQ (high QC).

For non-accuracy and precision validation runs, low, medium and high QCs may be analysed in duplicate. These QCs, along with the calibration standards, will provide the basis for the acceptance or rejection of the run.

- From separate primary stocks (or same if verified ± 5%)
- Single source of matrix
- A&P; 4 levels (LLOQ, LOW @ ≤ 3 x LLOQ, MID @ 30-50% of range & HIGH @ ≥ 75% of ULOQ) ❄
- Non-A&P; 3 levels @ LOW, MID & HIGH

ICH-M10 FAQ (22 May 2022).
How is the accurate preparation of the stock solution verified?

→

ICH-M10 FAQ (22 May 2022). By comparing two independently prepared stock solutions and demonstrating that the difference of measured response is within 5% difference = ([stock solution 1 – stock solution 2]) / (mean value) x 100.

3.2.5 Accuracy and Precision
3.2.5.1 Preparation of Quality Control Samples ★

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- Non-A&P; 3 levels @ LOW, MID & HIGH

Note, does not state [CAL] ≠ [QC]

ICH-M10 [illegible] indepen[illegible] tha[illegible] differ[illegible] value)[illegible]

Ends discussion on arithmetic vs geometric mid-point

3.2.5 Accuracy and Precision
3.2.5.1 Preparation of Quality Control Samples ★

3.2.5. Accuracy and precision

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3.2.5 Accuracy and Precision
3.2.5.1 Preparation of Quality Control Samples ★

L. Good that the mid-level QC concentration is well-defined now.

A. "MQC: around 30 - 50%". Is it allowed to set MQC around log-mid point of the curve?

A. Medium QC positioning, 30-50% of range is OK? Not the geometric mean?

- F… …or s…
- Single source of matrix
- A&P; 4 levels (LLOQ, LOW @ ≤ 3 x LLOQ, MID @ 30-50% of range & HIGH @ ≥ 75% of ULOQ) ❄
- Non-A&P; 3 levels @ LOW, MID & HIGH

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A. Guidance on the acceptable accuracy when comparing two stock solutions so that just 1 can be used for cals and QC not specified? There is reference to this in the FAQ but this is not an official document or linked from the ICH M10. How do we use the FAQ document?

ICH-M10 FAQ (22 May 2022). How is the accurate preparation of the stock solution verified?

ICH-M1… indepe… that the… differen… value) …

A. For non-accuracy and precision validation runs, low, medium and high QCs may be analysed in duplicate. These QCs, along with the calibration standards, will provide the basis for the acceptance or rejection of the run. Confusing that the word 'may' has been used. Does it mean that it is not mandatory to use QCs in these runs??

Discussion - Actions – proposal(Preparation of Quality Control Samples)

- Do we understand design and are we all clear on MID QC?
- Do we need clarity on how FAQ links to ICH-M10?

- Any other comments or questions?

3.2.5.2 Evaluation of Accuracy and Precision

3.2.5.2. Evaluation of accuracy and precision

Accuracy and precision should be determined by analysing the QCs within each run (within-run) and in different runs (between-run). Accuracy and precision should be evaluated using the same runs and data.

Within-run accuracy and precision should be evaluated by analysing at least 5 replicates at each QC concentration level in each analytical run. Between-run accuracy and precision should be evaluated by analysing each QC concentration level in at least 3 analytical runs over at least two days. To enable the evaluation of any trends over time within one run, it is recommended to demonstrate accuracy and precision of the QCs over at least one of the runs in a size equivalent to a prospective analytical run of study samples. Reported method validation data and the determination of accuracy and precision should include all results obtained, including individual QCs outside of the acceptance criteria, except those cases where errors are obvious and documented. Within-run accuracy and precision data should be reported for each run. If the within-run accuracy or precision criteria are not met in all runs, an overall estimate of within-run accuracy and precision for each QC level should be calculated. Between-run (intermediate) precision and accuracy should be calculated by combining the data from all runs.

The calibration curves for these assessments should be prepared using freshly spiked calibration standards in at least one run. If freshly spiked calibration standards are not used in the other runs, stability of the frozen calibration standards should be demonstrated.

The accuracy at each concentration level should be within ±15% of the nominal concentration, except at the LLOQ, where it should be within ±20%. The precision (%CV) of the concentrations determined at each level should not exceed 15%, except at the LLOQ, where it should not exceed 20%. For non-accuracy and precision validation runs, at least 2/3 of the total QCs and at least 50% at each concentration level should be within ±15% of the nominal values.

- A&P; 5 replicates, 4 levels, 3 Runs, > 1 day
- 1 @ robustness
- ≤ ± 15% (≤ ± 20% for A&P LLOQ)

- Non A&P; 2 replicates, 3 levels
- 2/3 (4/6) ≤ ± 15% (≥ 50% per level)

- Fresh CALS in at least one A&P run

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- Non A&P; 2 replicates, 3 levels
- 2/3 (4/6) ≤ ± 15% (≥ 50% per level)

- Fresh CALS in at least one A&P run

3.2.5.2 Evaluation of Accuracy and Precision

A. Is it OK to let one person do all 3 A&P runs?

A. Can QC samples for each validation run be stored upfront?

M. Add run with number of samples = max anticipated

A. CAL samples could be used frozen after the first run. Not in accordance with the FDA

A. Within-run accuracy and precision for each run. Does this means also validation runs with only 2 samples per concentration level prepared only for run-control?

A. To enable the evaluation of any trends over time within one run, it is recommended to demonstrate accuracy and precision of the QCs over at least one of the runs in a size equivalent to a prospective analytical run of study samples. Recommendation or mandatory? Should be covered with QCs at the end of each run?

A. Although the text is perfectly clear for CAL, there are no rules that define whether QCs for P&A should be freshly prepared or frozen QCs can be used (notice that stability data for frozen CAL or QCs are normally stablished later, or during the method setup in a exploratory manner)

➢ ≤ ± 15% (≤ ± 20% for A&P LLOQ)

A. Between-run precision and accuracy from all runs: what if more validation runs become necessary after some time, e.g. for determination of long term stability in matrix? Should it be re-evaluated every time when an addition validation run is performed (e.g. partial validation)?

➢

A. If the within-run accuracy or precision criteria are not met in all runs, an overall estimate of within- run accuracy and precision for each QC level should be calculated. Between-run (intermediate) precision and accuracy should be calculated by combining the data from all runs. [It is not clear to me what exactly we need to do based on this. It is great if we have some examples.]

Discussion - Actions – proposal(Evaluation of Accuracy and Precision)

- Do we understand design and that fresh calibration is used for at least one A&P run?
- Do we agree that its not specified that QCs need to be fresh (if frozen then we will need to demonstrate stability)?
- Do we understand design and value of the ‘full size’ robustness run?
- Do EBF need to provide examples of A&P calculations (within-day & between day)?

- Any other comments or questions?

3.2.6 Carryover

3.2.6. Carry-over

Carry-over is an alteration of a measured concentration due to residual analyte from a preceding sample that remains in the analytical instrument.

Carry-over should be assessed and minimised during method development. During validation carry-over should be assessed by analysing blank samples after the calibration standard at the ULOQ. Carry-over in the blank samples following the highest calibration standard should not be greater than 20% of the analyte response at the LLOQ and 5% of the response for the IS. If it appears that carry-over is unavoidable, study samples should not be randomised. Specific measures should be considered, validated and applied during the analysis of the study samples, so that carry-over does not affect accuracy and precision. This could include the injection of blank sample(s) after samples with an expected high concentration, before the next study sample.

- Double blank after ULOQ
- ≤ 20% of LLOQ response at RT
- ≤ 5% of ISTD response at RT

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- Double blank after ULOQ
- ≤ 20% of LLOQ response at RT
- ≤ 5% of ISTD response at RT

A. In how many runs the blank should be placed after the highest cal standard at the ULOQ?

A. What if the study samples have values above the ULOQ, what does this mean for your carry-over?

A. Can an alternative 'quantitative assessment be used; ULOQ, LLOQ, LLOQ (e.g. GBC; AAPS J. 2014 16(5) 885-93)?

L. Permits blanks to be injected between samples to minimize carryover.

3.2.7 Dilution Integrity ★

3.2.7. Dilution integrity

Dilution integrity is the assessment of the sample dilution procedure, when required, to confirm that it does not impact the accuracy and precision of the measured concentration of the analyte. The same matrix from the same species used for preparation of the QCs should be used for dilution.

Dilution QCs should be prepared with analyte concentrations in matrix that are greater than the ULOQ and then diluted with blank matrix. At least 5 replicates per dilution factor should be tested in one run to determine if concentrations are accurately and precisely measured within the calibration range. The dilution factor(s) and concentrations applied during study sample analysis should be within the range of the dilution factors and concentrations evaluated during validation. The mean accuracy of the dilution QCs should be within ±15% of the nominal concentration and the precision (%CV) should not exceed 15%.

In the cases of rare matrices, use of a surrogate matrix for dilution may be acceptable. It should be demonstrated that this does not affect precision and accuracy.

- DQC > [ULOQ]
- DQC ≥ [in-vivo]
- Same matrix & species
- ≥ 5 replicates per DF
- A&P ≤ ± 15%

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ICH-M10 FAQ (22 May 2022). In situations where a matrix is unavailable (eg shortage, 3Rs) can a similar surrogate matrix be used to dilute samples?

ICH-M10 FAQ (22 May 2022).
Yes, as long as the use of the surrogate matrix meets the requirements of the guideline, including accuracy, and precision, lack of interference, etc and the dilution quality control samples (QCs) are processed the same way. The rational needs to be well justified because the approach may be questioned

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- Same matrix & species
- ≥ 5 replicates
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Rare = 3Rs?

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3.2.7 Dilution Integrity ★

A. "At least 5 replicates per dilution factor should be tested in one run to determine if concentrations are accurately and precisely measured within the calibration range": not clear which is the meaning of replicates here and can lead to misunderstandings (it is not clearly established whether 5 independently diluted samples will be made for a specific dilution level, or whether a single diluted sample can be analysed in 5 replicates .)

L. Permits bracketing of dilution factors; assessment at each factor not required?

M. Maximum concentration used for dilution needs to be monitored during sample analysis. Previously it was showing you could dilute outside the range in using x fold

A. In the cases of rare matrices, use of a surrogate matrix for dilution may be acceptable. What is considered as rare matrix? All animals?

A. The dilution factor(s) and concentrations applied during study sample analysis should be within the range of the dilution factors and concentrations evaluated during validation. Could we conclude that a bracketing approach is fit for purpose (Validating the highest dilution factors).

A. If dilution factors of e.g. 5 and 10 have been tested, does this mean that a dilution factor of 2 cannot be used? Or are all dilution factors ≤10 allowed? (One can say that dilution factors of 1 to 10 have been tested.) Also for concentrations: Do the concentrations of the dilution QCs need to bracket the concentrations of the diluted samples? Bracketing is required in analytical runs, see section 3.3.2: The concentration of the dilution QCs should exceed that of the study samples being diluted (or of the ULOQ) and they should be diluted using the same dilution factor.

A. "The dilution factor(s)...should be within the range of the dilution factors and concentrations evaluated..." This would mean testing 100-fold dilution and then use 50-fold dilution for samples is not allowed as it is not within. Can the EBF comment on this.

Discussion - Actions – proposal (Dilution Integrity)

- Would you support using ‘surrogate matrix’ for ‘rare matrices’ (3Rs)?
- How do we understand design; **1 bulk, 5 diluted aliquots per DF and 1 replicate per diluted aliquot** (rather than 1 diluted aliquot and 5 replicates per DF)?
- Do we interpret 3.2.7 to mean validation of each dilution factor (e.g. 1:10, 1:50, 1:100, etc), bracketing lowest and highest dilution factor or **only at highest dilution factor and [DQC]?**
- Do we interpret 3.2.7 (and 3.3.2) to mean [DQC] ≥ [in-vivo]?

- Any other comments or questions?

3.2.8 Stability ★

3.2.8. Stability

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 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 matrix is evaluated using low and high concentration QCs. Aliquots of the low and high QCs are analysed at time zero and after the applied storage conditions that are to be evaluated. One bulk QC should be prepared at each concentration level. For each concentration tested, the bulk sample should be divided into a minimum of 3 aliquots that will be stored, stressed and analysed.

The QCs should be analysed against a calibration curve, obtained from freshly spiked calibration standards in a run with its corresponding freshly spiked 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 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.

For fixed dose combination products and specifically labelled drug regimens, the freeze-thaw, bench-top and long-term stability tests of an analyte in matrix should be conducted with the matrix spiked with all of the dosed compounds.

- ‘Bedside’ to ‘Bench’

- Two Levels (LOW & HIGH QC)
- + DQC level (if [in-vivo] > [ULOQ])
- ≥ 3 **aliquots** (not replicates) from **bulk**
- Minimum of 1 **replicate** per **aliquot**
- T_0 and T_{test} (independent variables) ❄
- **Fresh** calibration curve
- Run QC samples (3 levels, 2 reps)
- A @ ≤ ± 15%
- Fixed dose combination (FDC)

ICH-M10 FAQ (22 May 2022). What is the purpose of measuring the QC at time zero?

ICH-M10 FAQ (22 May 2022). To confirm the QCs were correctly prepared. Stability in the matrix (e.g. bench-top, long-term, freeze-thaw) should be evaluated by comparing with the nominal value

3.2.8 Stability ★

A. Would recommend combination stability experiment - the samples will undergo many different temperatures during the blood collection, shipment, analysis, re-analysis until storage

A. Does stability have to be done for each analyte, independently of its structure, and for each species?

A. Should the HQC or DQC concentration be increased if the concentrations of the study samples are consistently higher than the ULOQ?

M. Co-med stability required

A. Contradiction to 3.2.5.1. QC samples for run control should be prepared freshly?

L. The way we interpret the guidance we do not need to repeat stability assessment when we have done it once. ie if the method is transferred to a new laboratory validation reports for previous validation can be shared to document stability.

M. More matrix consumption: "For each concentration tested, the bulk sample should be divided into a minimum of 3 aliquots that will be stored, stressed and analysed."

A. 'Freshly spiked QCs or QCs for which stability has been proven'; should stability be proven in advance, or could this be shown later?

M. Stability in Dilution QC currently not assessed routinely

L. Good one bulk QC prep is clear

A. The sentence: "For fixed dose combination products and specifically labelled drug regimens, the freeze-thaw, bench-top and long-term stability tests of an analyte in matrix should be conducted with the matrix spiked with all of the dosed compounds." is hard to implement for a CRO since this is usually not knowledge shared with the CRO. The sentence also implies that validation work should be done AS LATE AS possible in order not to "miss" changes in combination products and similar.

A. Why must time zero be measured as the nominal concentration for evaluation of the stability?

A. Can a DQC cover the adopted HQC level for testing or is a re-validation of the method with a revised range required?

A. If study samples are expected to >ULOQ, do we include additional >ULOQ QC level along with H, M, L within range or just need to up the High QC to above the ULOQ?

3.2.8 Stability

3.2.8. Stability

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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 QCs that have been stored for a time that is equal to or longer than the study sample storage periods.

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For fixed dose combination products and specifically labelled drug regimens, the freeze-thaw, bench-top and long-term stability tests of an analyte in matrix should be conducted with the matrix spiked with all of the dosed compounds.

ICH-M10 FAQ (22 May 2022). What is the purpose of measuring the QC at time zero?

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- Minimum of 1 **replicate** per **aliquot**
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- **Fresh** calibration curve
- Run QC samples (3 levels, 2 reps)
- A @ ≤ ± 15%
- Fixed dose combination (FDC)

ICH-M10 FAQ (22 May 2022). To confirm the QCs were correctly prepared. Stability in the matrix (e.g. bench-top, long-term, freeze-thaw) should be evaluated by comparing with the nominal value

Discussion - Actions – Recommendations (Stability – Part 1)

- Do we interpret 3.2.8 to indicate stability does not need repeating if we have documented evidence (i.e. an approved & traceable BMV-compliant report)?
- Do we agree stability is performed for each analyte and species?
- Do we understand design; **1 bulk, 3 aliquots and 1 replicate** (vs independent variables i.e. time and temperature) and including 'time zero'?
- Do we agree that frozen run QC may be used (stability proven / will be proven)?
- Are we in agreement that [DQC] ≥ [in-vivo]?
- Do we agree that stability of FDC is 'special' and does not mean 'general' concomitant medication (co-med)?

- Any other comments or questions?

3.2.8.1 Stability (Freeze-Thaw)★

Freeze-thaw stability in matrix

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 QCs should be thawed and analysed according to the same procedures as the study samples. QCs should be kept frozen for at least 12 hours between the thawing cycles. 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.

- Two Levels (LOW & HIGH QC)
- **+ DQC level**
- ≥ 3 **aliquots**/level

- ≥ 12 h between F/T cycles
- ≥ 3 FT cycles ($T_{test} \geq T_{sample}$)
- QC cycles ≥ study sample cycles

- Fresh calibration curve
- Run QC samples (3 levels, 2 reps)❄

- A @ ≤ ± 15%

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A. F/T cycles - minimum of 3 cycles doesn't align with statement "The number of F/T cycles validated should equal or exceed the F/T cycles undergone by study samples

A. "QCs for freeze-thaw stability should be assessed using freshly prepared calibration standards and QCs, or QCs for which stability has been proven." leaves some unclarity. Is the phrase "freshly prepared calibration standards" actually not meant as "freshly spiked and prepared" as it was in previous guidelines? I also have heard the interpretation of "even stock solutions should be freshly prepared for such calibration standards, not only fresh spiking".

- Two Levels (LOW & HIGH QC)
- **+ DQC level**
- ≥ 3 **aliquots**/level

- ≥ 12 h between F/T cycles
- ≥ 3 FT cycles ($T_{test} \geq T_{sample}$)
- QC cycles ≥ study sample cycles

- Fresh calibration curve
- Run QC samples (3 levels, 2 reps)❄

- A @ ≤ ± 15%

3.2.8.1 Stability (Short-term)★

Bench-top (short-term) stability in matrix

Bench top matrix stability experiments should be designed and conducted to cover the laboratory handling conditions for the study samples.

Low and high 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., time from each freeze-thaw evaluation should not be added up).

- Two Levels (LOW & HIGH QC)
- **+ DQC level**
- ≥ 3 **aliquots**/level

- $T_{test} \geq T_{sample}$ (independent variables)
- Concurrent

- Fresh calibration curve
- Run QC samples (3 levels, 2 reps) ❄

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- **+ DQC level**
- ≥ 3 **aliquots**/level

- $T_{test} \geq T_{sample}$ (independent variables)
- Concurrent

- Fresh calibration curve
- Run QC samples (3 levels, 2 reps) ❄

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The total time on the bench top should be concurrent; it is not acceptable to use additive exposure to bench top conditions (i.e., time from each freeze-thaw evaluation should not be added up).

A. Bench-Top stability in matrix: Low and high QCs should be thawed in the same manner as the study samples and kept on the bench. Is it also allowed to store the samples first on bench-top and then freeze them till analysis?

- Two Levels (LOW & HIGH QC)
- **+ DQC level**
- ≥ 3 **aliquots**/level

- $T_{test} \geq T_{sample}$ (independent variables)
- Concurrent

- Fresh calibration curve
- Run QC samples (3 levels, 2 reps) ❄

- A @ ≤ ± 15%

3.2.8.1 Stability (Long-term)★

Long-term stability in matrix

The long-term stability of the analyte in matrix stored in the freezer should be established. Low and high QCs should be stored in the freezer under the same storage conditions and at least for the same duration as the study samples.

For chemical drugs, the stability at one temperature (e.g., -20°C) to can be extrapolated to lower temperatures (e.g., -70/-80°C).

For biological drugs, a bracketing approach can be applied, e.g., in the case that the stability has been demonstrated at -70/-80°C and at -20°C, then it is not necessary to investigate the stability at temperatures in between those two points at which study samples will be stored.

ICH-M10 FAQ (22 May 2022). For long-term stability, does a failed time-point mean you should not continue with longer time-points?

ICH-M10 FAQ (22 May 2022).
Additional time-points can be evaluated. Any failure should be investigated to identify the root cause and the impact on the stability assessment

- Two Levels (LOW & HIGH QC)
- **+ DQC level**
- ≥ 3 **aliquots**/level

- $T_{test} \geq T_{sample}$ (independent variables)
- Temperature extrapolation (Arrhenius) accepted for chemical drugs.
- Temperature bracketing for biological drugs (***def. drugs made by living organisms***)

- Fresh calibration curve
- Run QC samples (3 levels, 2 reps)❄
- A @ ≤ ± 15%

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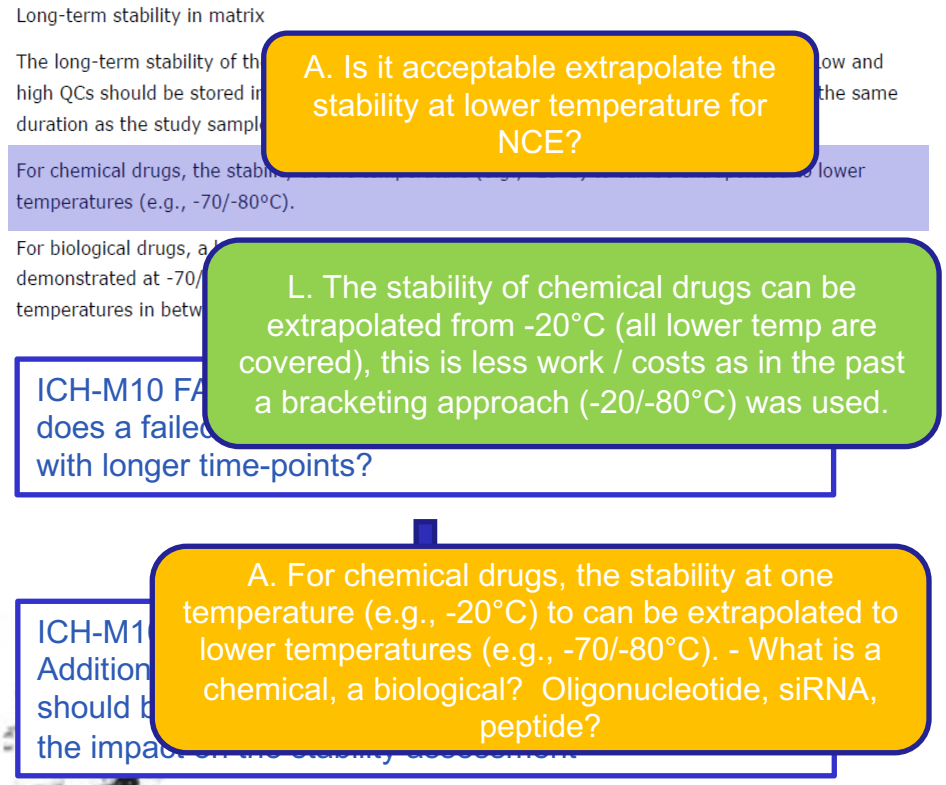
- Fresh calibration curve
- Run QC samples (3 levels, 2 reps)❄
- A @ ≤ ± 15%

3.2.8.1 Stability (Long-term)★



- Two Levels (LOW & HIGH QC)
- **+ DQC level**
- ≥ 3 **aliquots**/level

- $T_{test} \geq T_{sample}$ (independent variables)
- Temperature extrapolation (Arrhenius) accepted for chemical drugs.
- Temperature bracketing for biological drugs (***def. drugs made by living organisms***)

- Fresh calibration curve
- Run QC samples (3 levels, 2 reps)❄
- A @ ≤ ± 15%

3.2.8.2 Stability in Processed Samples ★

The stability of processed samples, including the time until completion of analysis (in the autosampler/instrument), should be determined. For example:

- Stability of the processed sample under the storage conditions to be used during the analysis of study samples (dry extract or in the injection phase)
- On-instrument/autosampler stability of the processed sample at injector or autosampler temperature.

The total time that a processed sample is stored must be concurrent (i.e., autosampler and other storage times cannot be added together).

- Two Levels (LOW & HIGH QC)
- **+ DQC level**
- ≥ 3 **aliquots**/level

- $T_{test} \geq T_{sample}$ (independent variables)
- Concurrent

- Fresh calibration curve
- Run QC samples (3 levels, 2 reps)
- A @ ≤ ± 15%

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- ≥ 3 **aliquots**/level

- $T_{test} \geq T_{samples}$ (independent variables)
- Concurrent

- Fresh calibration curve
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- Stability of the processed sample under the storage conditions to be used during the analysis of study samples (dry extract or in the injection phase)
- On-instrument/autosampler stability of the processed sample at injector or autosampler temperature.

The t… …osampler and other stora…

A. Processed sample stability. Not explicit when you start the stability clock and finish the stability clock

M. The stability of dry extracts is now requested. This is extra work in case of SPE methods or whenever the extract is dried down before reconstitution (which constitutes the 'extract' to be injected).

A. Sometime it is difficult to separate the stability, i.e. dry-extract stability is evaluated after the injection phase?

- Two Levels (LOW & HIGH QC)
- **+ DQC level**
- ≥ 3 …
- T_{tes} … les)
- Co…
- Fresh calibration curve
- Run QC samples (3 levels, 2 reps)
- A @ < + 15%

A. Stability of the analyte in processed samples - unclear whether reinjection viability may be applied as a measure of "stability" here. Requirements for stability of stored extracts using fresh prep standards and performance QCs remains unpopular and adds little value.

A. Wording around stability if the analyte in processed samples is not clearly communicated. Although can assume this is against a freshly extracted cal line and is conducted both on and off the autosampler?

3.2.8.3 Stability in Stock & Working Solutions★

3. Stability of the analyte and IS in stock and working solutions

The stability of the stock and working solutions of the analyte and IS should be determined under the storage conditions used during the analysis of study samples by using the lowest and the highest concentrations of these solutions. They should be assessed using the response of the detector. Stability of the stock and working solutions should be tested with an appropriate dilution, taking into consideration the linearity and measuring range of the detector. If the stability varies with concentration, then the stability of all concentrations of the stock and working solutions needs to be assessed. If no isotopic exchange occurs for the stable isotopically-labelled IS under the same storage conditions as the analyte for which the stability is demonstrated, then no additional stability determinations for the IS are necessary. If the reference standard expires, or it is past the retest date, the stability of the stock solutions made previously with this lot of reference standard are defined by the expiration or retest date established for the stock solution. The practice of making stock and working solutions from reference standards solely for extending the expiry date for the use of the reference standard is not acceptable.

- Analyte(s) & analogue Internal standard @ [low] and [high] (not stable label IS; if no isotope exchange)
- Stability in physical state (solid vs liquid) are independent
- T_{ref} vs T_{test} (independent variables)
- vs detector response (dilution into range if required)

- ≥ 3 **replicates**/[conc] (diluted)?
- A @ ≤ ± X%?

3.2.8.3 Stability in Stock & Working Solutions★

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Uncertainty on No. replicates and acceptance criteria

- Analyte(s) & analogue Internal standard @ [low] and [high] (not stable label IS; if no isotope exchange)
- Stability in physical state (solid vs liquid) are independent
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- vs detector response (dilution into range if required)

- ≥ 3 **replicates**/[conc] (diluted)?
- A @ ≤ ± X%?

3.2.8.3 Stability in Stock & Working Solutions★

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A. What does the sentence: "The practice of making stock and working solutions from reference standards solely for extending the expiry date for the use of the reference standard is not acceptable." exactly mean?

- Analyte(s) & analogue Internal standard @ [low] and [high] (not stable label IS; if no isotope exchange)
- T_{-x} vs T_{+x} (independent variables)
- …**replicates**/[conc] (diluted)?

A. It is not established, for example, that each stock solution stability evaluation needs to be initiated from a new weighing of a reference standard.

A. SS and WS stability: If stability varies with concentration, then the stability at each conc. level needs to assessed. However, it is not defined what degree of variability is acceptable.

A. Acceptance criteria for Stock and Working Solution stability are missing

M. In case stability testing for IS solutions is mandatory this causes additional work and higher costs as more reference material for IS is needed

A. It is not clear if a SS prepared prior to the ref material expiry date can be used, in case SS stability data exists. There is a clash in the content of the last two sentences in the same paragraph.

A. Stability determination of SIL-IS, what data should be presented?

3.2.8.4 Stability in Whole Blood ★

4. Stability of the analyte in whole blood

Sufficient attention should be paid to the stability of the analyte in the sampled matrix (blood) directly after collection from subjects and prior to preparation for storage to ensure that the concentrations obtained by the analytical method reflect the concentrations of the analyte in the subject's blood at the time of sample collection.

If the matrix used is plasma, the stability of the analyte in blood should be evaluated during method development (e.g., using an exploratory method in blood) or during method validation. The results should be provided in the Validation Report.

- Stability in blood to be assessed
- No specific assay; exploratory / method development
- No defined acceptance criteria
- T_{ref} vs T_{test} (independent variables)
- ≥ 3 **replicates**/[conc]?
- A @ ≤ ± X%?

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If the matrix used is plasma, the stability of the analyte in blood should be evaluated during method development (e.g., using an exploratory method in blood) or during method validation. The results should be provided in the Validation Report.

Uncertainty on No. replicates and acceptance criteria

Human only? 3Rs?

- Stability in blood to be assessed
- No specific assay; exploratory / method development
- No defined acceptance criteria
- T_{ref} vs T_{test} (independent variables)
- ≥ 3 **replicates**/[conc]?
- A @ ≤ ± X%?

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If the matrix used is plasma, the stability of the analyte in blood should be evaluated during method development (e.g., using an exploratory method in blood) or during method validation. The results should be provided in the Validation Report.

- Stability in blood to be assessed
- No specific assay; exploratory / method development
- No defined acceptance criteria
- T_{ref} vs T_{test} (independent variables)
- ≥ 3 **replicates**/[conc]?
- A @ ≤ ± X%?

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Sufficien…he sampled matrix (blood) directly after col… ensure that the concentrations obtained… analyte in the subject's blood at the time of sample collection.

If t…ethod dev…sults sho…

M. Blood stability should be provided in the validation report

A. If the analyte is analysed in serum, do we need to have a stability of analyte in whole blood?

A. It is enormously time and cost intensive to obtain fresh blood for testing stability in whole blood. Why should this be done for each method validation, even if a substance is well known?

M. i) much more work, ii) difficult to provide matrix, iii) cost of the matrix.

A. Are alternatives to exploratory blood methods acceptable for blood stability assessment?

A. Nonclinical: We traditionally run our GLP assays in-house and have traditionally not performed whole blood stability. Now we will need to find out how to access whole blood from all species. Or can we use 3R as argument that this is not necessary?

…od development

- No …
- T_{ref} …
- ≥ 3 **replicates**/[conc]?
- A @ ≤ ± X%?

A. Whole blood stability: Sufficient attention should be paid to the stability of the analyte in the sampled matrix (blood) directly after collection from subjects. Does "subject" mean it is only applicable for human matrix or also for animal?

A. Is stability in whole blood only required when plasma is used (i.e not serum)?

Discussion - Actions – Recommendations (Stability – Part 2)

- Do we agree that **'freshly prepared'** means 'fresh in matrix' not from freshly prepared primary solutions (SS & WS)?
- Do we accept design & acceptance criteria of 3 **replicates** and ± **X%** accuracy for **Stock And Working Solution Stability**?
- Do we accept design & acceptance criteria of 3 **replicates** and ± **X%** accuracy for **Blood Stability**?
- Do we agree to limit scope to human **Blood Stability** (or use human blood as surrogate of animal blood stability; 3Rs)?
- Do we understand **Blood Stability** is only applicable for plasma assay?

- Any other comments or questions?

3.2.9 Reinjection Reproducibility ★

3.2.9. Reinjection reproducibility

Reproducibility of the method is assessed by replicate measurements of the QCs and is usually included in the assessment of precision and accuracy. However, if samples could be reinjected (e.g., in the case of instrument interruptions or other reasons such as equipment failure), reinjection reproducibility should be evaluated to establish the viability of the processed samples and to support their storage prior to reinjection.

Reinjection reproducibility is assessed by reinjecting a run that is comprised of calibration standards and a minimum of 5 replicates of the low, middle and high QCs after storage. The precision and accuracy of the reinjected QCs establish the viability of the processed samples.

The results should be included in the Validation Report or provided in the Bioanalytical Report of the study where it was conducted.

- Assess viability of processed samples in event of delay by reinjecting a run
- Calibration curve
- ≥ 5 replicates @ 3 levels (L, M, H)
- $T_{test} \geq T_{sample}$ (independent variables)
- A&P @ ≤ ± 15%

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... vided in the Bioanalytical Report of the ...

L. This is clear and doesn't need fresh calibration samples to be prepared.

A. Re-injection reproducibility evaluation is not clearly described

A. Not so clear how we differentiate between re-injection reproducibility and processed sample stability

M. Perform processed sample stability (autosampler stability) and reinjection reproducibility.

A. What are the acceptance criteria? I assume +-15%?

- Calibration c...
- ≥ 5 replicates @ 3 levels (L, M, H)
- $T_{test} \geq T_{sample}$ (independent variables)
- A&P @ ≤ ± 15%

M. Additional run to cover storage period on autosampler

A. No mention of LLoQ QC?

A. Are the reinjected QCs assessed against the original CAL stds or the re-injected CAL standard?

A. storage already covered by autosampler stability?

3.3 Study Sample Analysis

3.3. Study sample analysis

The analysis of study samples can be carried out after validation has been completed, however, it is understood that some parameters may be completed at a later stage (e.g., long-term stability). By the time the data are submitted to a regulatory authority, the bioanalytical method validation should have been completed. The study samples, QCs and calibration standards should be processed in accordance with the validated analytical method. If system suitability is assessed, a predefined specific study plan, protocol or SOP should be used. System suitability, including apparatus conditioning and instrument performance, should be determined using samples that are independent of the calibration standards and QCs for the run. Subject samples should not be used for system suitability. The IS responses of the study samples should be monitored to determine whether there is systemic IS variability. Refer to Table 1 for expectations regarding documentation.

- Analysis of study samples using ‘validated assay’
- SST is predefined
- SST does not use CAL or QC destined for the run
- SST does not use study samples
- Monitor IS response to identify systemic variability in each run

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the calibration standards
ity. The IS responses of
mic IS variability. Refer to

A. It is not clear whether SST is needed or not ('if system suitability is assessed'…)

A. It is not clear how the systemic IS variability should be evaluated. Also, there are no acceptance criteria.

A. IS monitoring is unfortunately not specified more in detail and no criteria are defined. Also a distinction between "structurally similar analogues" used as internal standard and a stable isotope labelled IS would be useful. Latter obviously compensates much better any variations of analyte response or limited recovery of the analyte compared to the first class of internal standards

- Ana sing 'val
- SST is prec
- SST does n destined for
- SST does not use study samples
- Monitor IS response to identify systemic variability in each run

M. SST now a separate spiked sample independent from others.

M. Documented monitoring of IS response needs to be performed

Ref. Bioanalysis (2014) 6(20) 2767-2774

3.3.1 Analytical Run ★

3.3.1. Analytical run

An analytical run consists of a blank sample (processed matrix sample without analyte and without IS), a zero sample (processed matrix with IS), calibration standards at a minimum of 6 concentration levels, at least 3 levels of QCs (low, medium and high) in duplicate (or at least 5% of the number of study samples, whichever is higher) and the study samples to be analysed. The QCs should be interspersed in the run in such a way that the accuracy and precision of the whole run is ensured. Study samples should always be bracketed by QCs.

The calibration standards and QCs should be spiked independently using separately prepared stock solutions, unless the accuracy and stability of the stock solutions have been verified. All samples (calibration standards, QCs and study samples) should be processed and extracted as one single batch of samples in the order in which they are intended to be analysed. Analysing samples that were processed as several separate batches in a single analytical run is discouraged. If such an approach cannot be avoided, for instance due to bench top stability limitations, each batch of samples should include low, medium and high QCs.

For comparative BA/BE studies, it is advisable to analyse all samples of one subject together in one analytical run to reduce variability.

The impact of any carry-over that occurs during study sample analysis should be assessed and reported (refer to Section 3.2.6). If carry-over is detected, its impact on the measured concentrations should be mitigated (e.g., non-randomisation of study samples, injection of blank samples after samples with an expected high concentration) or the validity of the reported concentrations should be justified in the Bioanalytical Report.

- CAL @ ≥ 6 levels
- QC @ 3 levels (L, M, H) in duplicate and ≥ 5% of study samples
- Bracket study samples with QC

- Separate primary stock for CAL & QC (unless verified)
- Independently prepared CAL and QC samples
- CALs, QCs and samples processed as ‘batch’
- BA/BE advisory
- Assess carryover in each ‘batch’

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Study sam

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The impact of any car
reported (refer to Sec
should be mitigated (e
samples with an expe
justified in the Bioana

A. Impact of any carry-over: no criteria of acceptability in the guideline. Is there consensus on the maximum impact limit to be accepted .

A. Does a batch = a plate? What if individual tubes are used; does a batch = a rack?

A. What does bracketed mean, can there be a set of three QCs directly after the calibration curve and one set of three QCs at the end of the run?

A. "Analysing samples that were processed as several separate batches in a single analytical run is discouraged. If such an approach cannot be avoided, for instance due to bench top stability limitations, each batch of samples should include low, medium and high QCs." Do you avoid separate batches in one analytical run by all means? What was done in the past ?.

L. Batch vs run acceptance permitted

- QC @ 3 levels (L, M, H) in duplicate
- Bracket study samples with QC
- S & QC (u
- so 'batch'

M. Carryover in sample analysis batches must be reported

M. When samples are analysed in several batches due to a short bench top stability, we currently include 2 QC samples but not 3 as required in the ICH M10.

A. Unclear if a run consisting of two 96-well plates is now considered a single batch of samples, if the plates are processed simultaneously. If not, running multiple plates is apparently discouraged

A. Analysing samples that were processed as several separate batches in a single analytical run is discouraged. Does "batch" mean different times of sample work up, or more than one deep well plate?

Discussion - Actions – Recommendations (Analytical Run)

- Do we interpret bracketing to mean at a minimum ‘QC – Samples – QC’?
- Do we agree that several ‘batches’ can form a ‘run’ and that QCs (duplicate at three levels) are needed in each ‘batch’?
- Do we need further discussion on carry-over, or do we already understand impact and how to mitigate it?

- Any other comments or questions?

3.3.2 Acceptance Criteria for an Analytical Run (1)

3.3.2. Acceptance criteria for an analytical run

Criteria for the acceptance or rejection of an analytical run should be defined in the protocol, in the study plan or in an SOP. In the case that a run contains multiple batches, acceptance criteria should be applied to the whole run and to the individual batches. It is possible for the run to meet acceptance criteria, even if a batch within that run is rejected for failing to meet the batch acceptance criteria. Calibration standards in a failed batch cannot be used to support the acceptance of other batches within the analytical run.

The back-calculated concentrations of the calibration standards should be within ±15% of the nominal value, except for the LLOQ for which it should be within ±20%. At least 75% of the calibration standard concentrations, which should include a minimum of six concentration levels, should fulfil these criteria. If more than six calibration standard levels are used and one of the calibration standards

does not meet the criteria, this calibration standard should be rejected and the calibration curve without this calibration standard should be re-evaluated and a new regression analysis performed.

If the rejected calibration standard is the LLOQ, the new lower limit for this analytical run is the next lowest acceptable calibration standard of the calibration curve. This new lower limit calibration standard will retain its original acceptance criteria (i.e., ±15%). If the highest calibration standard is rejected, the ULOQ for this analytical run is the next acceptable highest calibration standard of the calibration curve. The revised calibration range should cover at least 3 QC concentration levels (low, medium and high). Study samples outside of the revised range should be reanalysed. If replicate calibration standards are used and only one of the LLOQ or ULOQ standards fails, the calibration range is unchanged.

At least 2/3 of the total QCs and at least 50% at each concentration level should be within ±15% of the nominal values. If these criteria are not fulfilled the analytical run should be rejected. A new analytical batch should be prepared for all study samples within the failed analytical run for subsequent analysis. In the cases where the failure is due to an assignable technical cause, samples may be reinjected.

- Cal; A @ ≤ ±15% (± 20% at LLOQ)
- 75% of all standards and ≥ 6 levels
- ≥ 50% / level for replicates

- QC; A @ ≤ ±15% (L, M, H), 2/3 (4/6) and ≥ 50% per level

- If CAL and/or QC criteria are not fulfilled, the analytical run is rejected

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At least [illegible]% of the [illegible] qu[illegible] an[illegible] r[illegible]

- Cal[illegible] LOQ)
- [illegible]s
- QC; A @ ≤ ±[illegible]% (L, M, H), 2/3 (4/6) and ≥ 50% per level
- If CAL and/or QC criteria are not fulfilled, the analytical run is rejected

Caveat for multiple ‘batches’ to make a ‘run’

Usual caveats for failing LLOQ or ULOQ CAL standards

There is no ‘valid’ sample concentration data from a failed run

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- Cal; A @ ≤ ±15% (± 20% at LLOQ)
- 75% of all standards and ≥ 6 levels
- ≥ 50% / level for replicates

- QC; A @ ≤ ±15% (L, M, H), 2/3 (4/6) and ≥ 50% per level

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T… in ±15% of the nominal v… of the calibration standard concentrations, which should include a minimum of six concentration levels, should fulfil these criteria. If more than six calibration standard levels are used and one of the calibration standards c… m… calibration standards are used and only one of the LLOQ or ULOQ standards fails, the calibration range is unchanged.

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L. Permits dropping LLOQ and ULOQ and adjusting curve

A. 1st paragraph Not very clearly written - for example: It is possible for the run to meet acceptance criteria even if a batch within that run is rejected for failing to meet the batch acceptance criteria. What is the difference between a run and a batch in this context?

- Cal; A @ ≤ ±15% (± 20% at LLOQ)
- 75% of all standards and ≥ 6 levels
- ≥ 50% / level for replicates

- QC; A @ ≤ ±15% (L, M, H), 2/3 (4/6) and ≥ 50% per level

- If CAL and/or QC criteria are not fulfilled, the analytical run is rejected

3.3.2 Acceptance Criteria for an Analytical Run (2)

Analytical runs containing samples that are diluted and reanalysed should include dilution QCs to verify the accuracy and precision of the dilution method during study sample analysis. The concentration of the dilution QCs should exceed that of the study samples being diluted (or of the ULOQ) and they should be diluted using the same dilution factor. If multiple dilution factors are used in one analytical run, then dilution QCs need only be diluted by the highest and lowest dilution factors. The within-run acceptance criteria of the dilution QC(s) will only affect the acceptance of the diluted study samples and not the outcome of the analytical run.

When several analytes are assayed simultaneously, there should be one calibration curve for each analyte studied. If an analytical run is acceptable for one analyte but has to be rejected for another analyte, the data for the accepted analyte should be used. The determination of the rejected analyte requires re-extraction and analysis only for the analyte that is reanalysed. Only data for this reanalysed analyte needs to be reported.

The back-calculated concentrations of the calibration standards and QCs of passed and accepted runs should be reported. The overall (between-run) accuracy and precision of the QCs of all accepted runs should be calculated at each concentration level and reported in the analytical report (refer to Section 8 Documentation and Table 1). If the overall mean accuracy and/or precision fails the 15% criterion, an investigation to determine the cause of the deviation should be conducted. In the case of comparative BA/BE studies, it may result in the rejection of the data.

- Use validated dilution (3.2.7)
- [DQC] ≥ [in-vivo] (or of ULOQ)
- Use same dilution factor (for one)
- Use lowest and highest dilution factors (for multiple)
- Within run acceptance of DQC only affects diluted study sample results
- ≥ 2 aliquots per DF or two **replicates** per DF (≥ 50% pass per DF)?
- A @ ≤ ± 15%?

- Multiple analyte acceptance
- Overall (between-run) A&P ≤ ± 15% (of accepted runs)

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The back-calculated concentrations of the calibration standards and QCs of passed and accepted runs should be reported. The overall (between-run) accuracy and precision of the QCs of all accepted runs should be calculated at each concentration level and reported in the analytical report (refer to Section 8 Documentation and Table 1). If the overall mean accuracy and/or precision fails the 15% criterion, an investigation to determine the cause [illegible] comparative BA/BE

No. DQC replicates & acceptance criteria; inferred from Run QC description?

- Use validated dilution (3.2.7)
- [DQC] ≥ [in-vivo] (or of ULOQ)
- Use same dilution factor (for one)
- Use lowest and highest dilution factors (for multiple)
- Within run acceptance of DQC only affects diluted study sample results
- ≥ 2 aliquots per DF or two **replicates** per DF (≥ 50% pass per DF)?
- A @ ≤ ± 15%?

- Multiple analyte acceptance
- Overall (between-run) A&P ≤ ± 15% (of accepted runs)

EBF

3.2.2 Acceptance Criteria for an Analytical Run (2)

A. How many replicates (3 or 5 or?) need to be tested for each dilution factor?

A. What should be done if using bracketing approach and one DQC level fails?

L. Including only the lowest and highest dilution factors for dilution QCs may be slightly less work, since we now include all dilution factors used

A. At what concentration the dilution QCs are to be prepared, a predefined concentration (e.g., the one tested in validation or defined in the study protocol) or at a concentration selected during sample analysis, based on the concentration of the study samples to be diluted?

M. Dilution QCs: these should not be prepared/analyzed if the dil. integrity has been proven during validation. Overall mean accuracy or precision failing (but individual runs passing) will be additional work to investigate the root cause

A. Does this mean that we always need to have dilution QC(s) if unknown samples are diluted in the analytical run even though the dilution factor(s) were validated in the method validation? Why we have the definition only in the chromatography section and not in LBA?]

M. Extra dilution QCs with all dilution factors used cause more work; however this pushes in direction of using one dilution factor per analytical run only in order to avoid the extra work. In some cases (non-clinical study with potentially more samples to be diluted) samples therefore most likely will be diluted much more than actually needed; if this then makes sense is somehow questionable.

No. DQC replicates

M. It seem as they missed to include this sentence from section 3.2.7 in the final document "The dilution factor(s) and concentrations applied during study sample analysis should be within the range of the dilution factors and concentrations evaluated during validation" We had hoped that dilutional QCs would not be needed during sample analysis if dilutional factor had been tested and accepted during validation

A. Dilution QCs not included in production runs?

M. dilution QCs need to reflect concentration of the samples that need to be diluted.

M. Routine use of dilution QC for lowest and highest dilution will reduce throughput

Discussion - Actions – Recommendations (Acceptance Criteria for Analytical Run – Dilution QC)

- Do we understand this to mean ≥ 2 **replicates** or **aliquots** per DF?
- Do we accept acceptance criteria; ≥ 50% pass & A @ ≤ ± 15%?
- Do we agree that we bracket the lowest and highest Dilution Factors with DQCs?
- Do we agree and accept that [DQC] ≥ [in-vivo]
- Do we understand potential implications if [in-vivo] ≥ [DQC] (3.2.8 Stability)?
- Do we agree that if a Dilution Factor (DQC) 'bracket' fails, only samples diluted with the passing Dilution Factor (DQC) are accepted?

- Any other comments or questions?

3.3.3 Calibration Range

3.3.3. Calibration range

If a narrow range of analyte concentrations of the study samples is known or anticipated before the start of study sample analysis, it is recommended to either narrow the calibration curve range, adapt the concentrations of the QCs, or add new QCs at different concentration levels as appropriate, to adequately reflect the concentrations of the study samples.

At the intended therapeutic dose(s), if an unanticipated clustering of study samples at one end of the calibration curve is encountered after the start of sample analysis, the analysis should be stopped and either the standard calibration range narrowed (i.e., partial validation), existing QC concentrations revised, or QCs at additional concentrations added to the original curve within the observed range before continuing with study sample analysis. It is not necessary to reanalyse samples analysed before optimising the calibration curve range or QC concentrations.

The same applies if a large number of the analyte concentrations of the study samples are above the ULOQ. The calibration curve range should be changed, if possible, and QC(s) added or their concentrations modified. If it is not possible to change the calibration curve range or the number of samples with a concentration above the ULOQ is not large, samples should be diluted according to the validated dilution method.

At least 2 QC levels should fall within the range of concentrations measured in study samples. If the calibration curve range is changed, the bioanalytical method should be revalidated (partial validation) to verify the response function and to ensure accuracy and precision.

- Change calibration range
- Revise / add QC levels

- At least 2 QC levels should fall within range of study samples (i.e. L/M or M/H)

ICH-M10 FAQ (22 May 2022). When adding a new QC concentration level during study sample analysis without changing the calibration curve range, is it necessary to validate the new QC concentration level with a partial validation?

ICH-M10 FAQ (22 May 2022).
The precision and accuracy of the new concentration level should be demonstrated before use in study sample analysis. This can be documented either as a partial validation or a note to the bioanalytical report

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At least 2 QC levels should fall within the range of concentrations measured in study samples. If the calibration curve range is changed, the bioanalytical method should be revalidated (partial validation) to verify the response function and to ensure accuracy and precision.

- Intended **therapeutic** dose (i.e. clinical Phase II/III & pivotal BA/BE?)
- Change calibration range
- Revise / add QC levels
- At least 2 QC levels should fall within range of study samples (L/M or M/H)

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ULOQ. The calibration curve range
concentrations modified. If it is no of
samples with a concentration above the g to the
validated dilution method.

At least 2 QC levels should fall within the range of concentrations measured in study samples. If the calibration curve range is changed, the bioanalytical method should be revalidated (partial validation)

This is key driver and may limit scope of any changes

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  or M/R)

M. Potentially more work and time consuming is the outcome of following sentences: "At the intended therapeutic dose(s), if an unanticipated clustering of study samples at one end of the calibration curve is encountered after the start of sample analysis, the analysis should be stopped and either the standard calibration range narrowed (i.e., partial validation), existing QC concentrations revised, or QCs at additional concentrations added to the original curve within the observed range before continuing with study sample analysis."

A. What does large number mean when thinking about redefining the range. 10%?

ISR M10 FAQ (22 May 2022): When adding a new QC concentration level during study sample analysis without changing the calibration curve range, is it necessary to validatye the new QC concentration level with a partial validation?

A. At least 2 QC sample levels should fall within the range of concentrations measured in study samples" Should we interpret this as two additional QCs in additional to the standard QCs

ntration
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ther as a
partial validation or a note to the bioanalytical report

3.3.4 Reanalysis of Study Samples (1)

3.3.4. Reanalysis of study samples

Possible reasons for reanalysis of study samples, the number of replicates and the decision criteria to select the value to be reported should be predefined in the protocol, study plan or SOP, before the actual start of the analysis of the study samples. For study samples in which multiple analytes are being analysed, a valid result for one analyte should not be rejected if the other analyte fails the acceptance criteria.

The number of samples (and percentage of total number of samples) that have been reanalysed should be reported and discussed in the Bioanalytical Report. For comparative BA/BE studies, a separate table should report values from rejected runs.

Some examples of reasons for study sample reanalysis are:

- Rejection of an analytical run because the run failed the acceptance criteria with regard to accuracy of the calibration standards and/or the precision and accuracy of the QCs
- IS response significantly different from the response for the calibration standards and QCs (as pre-defined in an SOP)
- The concentration obtained is above the ULOQ
- The concentration observed is below the revised LLOQ in runs where the lowest calibration standard has been rejected from a calibration curve, resulting in a higher LLOQ compared with other runs
- Improper sample injection or malfunction of equipment
- The diluted study sample is below the LLOQ
- Identification of quantifiable analyte levels in pre-dose samples, control or placebo samples
- Poor chromatography (as pre-defined in an SOP)

For comparative BA/BE studies, reanalysis of study samples for a PK reason (e.g., a sample concentration does not fit with the expected profile) is not acceptable, as it may bias the study result.

- Defined *a priori* (e.g. Plan or SOP)
- No. replicates & decision criteria
- Multiple analytes
- Reporting of reassay
- Caveat for BA/BE study (PK reassay)

3.3.4 Reanalysis of Study Samples (1)

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Possible reasons for reanalysis of study samples, the number of replicates and the decision criteria to ...or SOP, before the ...ltiple analytes are ...analyte fails the

M. For comparative BA/BE studies, a separate table should report values from rejected runs.

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- IS response significantly different from the response for the calibrat... ...ards and QCs (as pre-defined in an SOP)
- The con...
- Th... ...ith
- ...ebo samples
- Poor chrom...

There is no valid sample concentration data from a rejected run

For comparative BA/BE studies, reanalysis of study samples for a PK reason (e.g., a sample concentration does not fit with the expected profile) is not acceptable, as it may bias the study result.

- Defined *a priori* (e.g. Plan or SOP)
- No. replicates & decision criteria
- ...

M. For comparative BA/BE studies, a separate table should report values from rejected runs. More work to report these.

- Reporting of reassay
- ...

A. Unfortunately no guidance on following sentence: "IS response significantly different from the response for the calibration standards and QCs (as pre-defined in an SOP)

Ref. Bioanalysis (2014) 6(20) 2767-2774

3.3.4 Reanalysis of Study Samples (2)

For comparative BA/BE studies, reanalysis of study samples for a PK reason (e.g., a sample concentration does not fit with the expected profile) is not acceptable, as it may bias the study result.

Any reanalysed samples should be identified in the Bioanalytical Report and the initial value, the reason for reanalysis, the values obtained in the reanalyses, the final accepted value and a justification for the acceptance should be provided. Further, a summary table of the total number of samples that have been reanalysed for each reason should be provided. In cases where the first analysis yields a non-reportable result, a single reanalysis is considered sufficient (e.g., concentration above the ULOQ or equipment malfunction). In cases where the value needs to be confirmed (e.g., pre-dose sample with measurable concentrations) replicate determinations are required if sample volume allows.

The safety of trial subjects should take precedence over any other aspect of the trial. Consequently, there may be other circumstances when it is necessary to reanalyse specific study samples for the purpose of a safety investigation.

- Caveat for BA/BE study (PK reassay)
- Reporting of reassay (initial value, reason, reassay value, final value)
- GCP (patient safety) takes precedence for clinical bioanalysis

3.3.4 Reanalysis of Study Samples (2)

For comparative BA/BE studies, reanalysis of study samples for a PK reason (e.g., a sample concentration does not fit with the expected profile) is not acceptable, as it may bias the study result.

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The safety of trial subjects should take precedence over any other aspect of the trial. Consequently, ther… pu… samples for the

A. If reanalysis of samples for PK reasons is thought to bias BA/BE study results surely the same applies to any study!

A. Reporting of results when replicate reanalysis is needed is not defined!

There is no valid sample
co…
re…

A. Identification of quantifiable analyte levels in pre-dose samples, control or placebo samples - not currently covered in local SOP. Spurious repeats that have no analytical root cause are avoided for the risk of picking and choosing data.

- Caveat for BA/BE study (PK…

A. Pre-dose samples are not always necessarily true pre-dose sample so this can be misinterpreted. Should be clarified.

- Reporting of reassay (initial value, rea… …e)

M. Additional table required summarising number of repeats per reason

- GCP (patient safety) takes prece… …sis

M. A summary table of the total number of samples that have been reanalysed for each reason should be provided

Discussion - Actions – Recommendations (Reanalysis of Study Samples)

- Do we agree that there are no bioanalytically ‘valid’ study sample concentrations obtained from a failed run?
- Any other comments or questions?

3.3.5 Reinjection of Study Samples

3.3.5. Reinjection of study samples

Reinjection of processed samples can be made in the case of equipment failure if reinjection reproducibility has been demonstrated during validation or provided in the Bioanalytical Report where it was conducted. Reinjection of a full analytical run or of individual calibration standards or QCs simply because the calibration standards or QCs failed, without any identified analytical cause, is not acceptable.

- Documented reinjection reproducibility (3.2.9)
- Only with identified analytical cause

3.3.6 Integration of Chromatograms

3.3.6. Integration of chromatograms

Chromatogram integration and reintegration should be described in a study plan, protocol or SOP. Any deviation from the procedures described *a priori* should be discussed in the Bioanalytical Report. The list of chromatograms that required reintegration, including any manual integrations, and the reasons for reintegration should be included in the Bioanalytical Report. Original and reintegrated chromatograms and initial and repeat integration results should be kept for future reference and submitted in the Bioanalytical Report for comparative BA/BE studies.

Reintegration Definition (9. Glossary); Change of the original integration of a chromatographic peak

- Defined *a priori* (e.g. Plan or SOP)
- Reporting (all studies)
  - List of chromatograms that required reintegration
  - Manual integration
  - Reason for reintegration
- Retain
  - Original and reintegrated chromatograms
  - Plus reported for BA/BE

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3.3.6 Integration of Chromatograms

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M. The list of chromatograms that required reintegration, including any manual integrations, and the reasons for reintegration should be included in the Bioanalytical Report. More work to report these.

M. List of reintegrated chromatograms needed in the report. Is this just for BE studies?

Reintegration Definition (9. Glossary); Change of the original integration of a chromatographic peak

M. More work for documentation group writing the reports: "Original and reintegrated chromatograms and initial and repeat integration results should be kept for future reference and submitted in the Bioanalytical Report for comparative BA/BE studies."

priori (e.g. Plan or SOP)

- Reporting (all studies)

A. Is reintegration if one parameter is (e.g. bunching factor or minimum area) modified for the whole batch?

A. Reintegration not defined

Ref GBC; AAPS J. 2014 16(5) 885-93

M. Need to submit original and reintegrated chromatograms in reports for BA/BE studies

Discussion - Actions – Recommendations (Integration of Chromatograms)

- Any other comments or questions?

Concluding Discussion - Actions - Recommendations

- Any other comments or questions?

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- EBF community that contributed to discussion on ICH-M10 and provided feedback survey

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