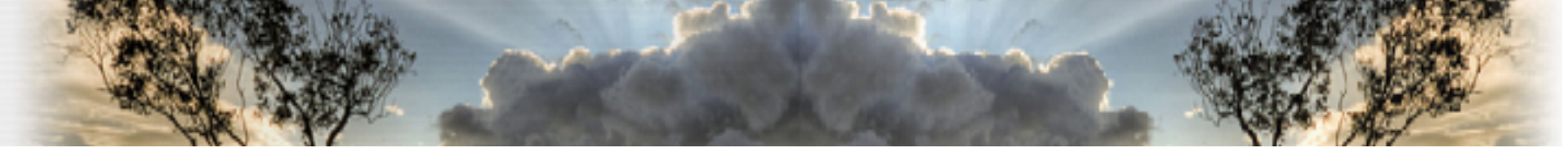

Guideline paragraphs anticipated of not needing a discussion

Johannes Stanta, on behalf of the EBF

The following topics are discussed in plenary sessions:

- **Scope**
- **Method development**
- **Full & partial validation**
- **Cross-validation**
- **Stability testing**
  - Benchtop & freeze/thaw
  - Blood stability
  - Long term
- **ISR**
- **Documentation & Glossary**
- **Repeat analysis**

Topics covered in parallel sessions

- Dried Matrix Methods
- New or Alternative Technologies
- Commercial and Diagnostic Kits

If you feel we have not covered sometime important in the Focus Workshop, or we did not cover a specific chromatography topics in enough detail for one of the common topics, please speak up during the **panel discussions** at the end of each break-out session…

Paragraphs from ICH M10

- The following chromatography paragraphs will **not** need a detailed discussion:
  - Calibration curve and range
  - Acceptance Criteria for an Analytical Run
  - (Re)integration of chromatograms
  - Carry-over
  - Recovery

3.2.4 Calibration Curve and Range

- Cals should be prepared in the same biological matrix as the study samples.
- Blank, zero sample and at least 6 concentration levels of calibration standards
- A simple regression model should be used.
- The regression model, weighting scheme and transformation determined during method validation.
- The back-calculated concentrations of the calibration standards should be presented together with the calculated mean accuracy values. All acceptable curves obtained during validation, based on a minimum of 3 independent runs over several days, should be reported.
- 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.
- The Cals 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.

3.2.5.2 Evaluation of Accuracy and Precision

- A&P should be determined by analysing the QCs within each run (within-run) and in different runs (between-run).
- Within-run A&P should be evaluated by analysing at least **5 replicates** at each QC concentration level in each analytical run.
- Between-run A&P should be evaluated by analysing each QC concentration level in at least **3 analytical runs over at least two days**.
- One of the runs in a **size equivalent to a prospective analytical run** of study samples.
- Reported method validation data and the determination of A&P should **include all results obtained**, including individual QCs outside of the acceptance criteria, except those cases where errors are obvious and documented.

3.2.5.2 Evaluation of Accuracy and Precision

- Within-run A&P 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 overall 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%**.

3.3.2 Acceptance Criteria for an Analytical Run

- Criteria 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.
- 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, with a minimum of six concentration levels, should fulfil these criteria**. If more than 6 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.

3.3.2 Acceptance Criteria for an Analytical Run

- 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 needs to 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.

3.3.2 Acceptance Criteria for an Analytical Run

- 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. 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 a re-extracted analytical batch and analysis.

3.3.2 Acceptance Criteria for an Analytical Run

- 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.**
- **If the overall mean accuracy 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.

A&P - EBF position on the subject

- Toward decision-based acceptance criteria for Bioanalytical Method Validation: a proposal for discussion from the European Bioanalysis Forum
  - Bioanalysis (2018) 10(16): 1255–1259

https://www.future-science.com/doi/10.4155/bio-2018-0131

- EBF Focus Workshop, Lisbon 2017:
  - Steve White, Run Acceptance Criteria

http://www.e-b-f.eu/wp-content/uploads/2018/06/fw201709-11.-Steve-White-Runacceptance-criteria.pdf

From EBF Focus Workshop, Lisbon 2017: Run Acceptance Criteria

Summary

- Chromatographic vs. LBA acceptance criteria invites ambiguity over utilization of alternate ("hybrid") platforms
- Currently different criteria for different platforms whilst the (PK) data is used for the same purpose
- There is now an opportunity to challenge this paradigm
  - or live with the consequences for the next 20+ years

Feedback from EBF Strategy (from Hasselt) and delegate survey

The overall (between-run) accuracy and precision of the QCs of all accepted runs should be calculated

You may have one outlier with no provable assignable cause which will push CV > 15%. At end of study its too late.

Very clear, just consider that between-run precision is not the overall. Between run is the residual variability between the runs. Overall is the sample population variability. It is suggested to remove the (between-run) in brackets.

What is the meaning of "an overall estimate of within-run accuracy". It's not clear.

3.2.6. Carry over

- 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, tested during the validation 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.

in line with Current guidelines

Feedback from EBF Strategy and delegates

/* NO COMMENT */

EBF position on the subject

Although carry over assessment will likely be part of method development, EBF recommends to document carry over only for sample analysis. EBF does not provide nominal criteria to evaluate carry over, but would expect a scientific evaluation to ensure any undue carry over does not impact the decisions taken from the data. These criteria can be different depending on study type. In contrast to other acceptance criteria, carry over assessment would be allowed post analysis, but documentation should allow scientific scrutiny.

Tiered approach into practice: scientific validation for chromatography-based assays in early development – a recommendation from the European Bioanalysis Forum

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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.

FDA Guideline 2018

An SOP or guideline for sample data reintegration for CCs should be established a priori. This SOP or guideline should define the criteria for re-integration and how the re-integration will be performed.

The rationale for the re-integration should be clearly described and documented.

Audit trails should be maintained.

Original and re-integrated data should be **documented and reported**.

Valuable literature

- **Manual chromatographic baseline integration: is it needed, if so when should it be used?**

Howard M Hill, David Bakes, Iain Love

Bioanalysis, 2014

Feedback from EBF Strategy and delegates

[…] initial and the final integration data should be documented at the laboratory and **should be available upon request.**

Need to carefully define 'integration' and 'reintegration' and would strongly suggest GBC 3S paper. Please note that a LIMS system cannot have two 'valid' derived concentration results, so it may well be impossible to submit concentration data for 'initial' and 'repeat' integrations for BA/BE studies in report (but original and repeat chromatograms and integrated peaks areas can be retained).

[…] The list of chromatograms that required reintegration, including any manual integrations, and the reasons for reintegration should be **documented**. […].

This should only be done for BA/BE studies

Why is this listing needed? The issue of reporting original and reintegrated chromatograms in BA/BE studies also needs to be revisited.

7.3 Recovery

- For methods that employ sample extraction, the recovery (extraction efficiency) should be evaluated. Recovery is reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method.
- Recovery is determined by comparing the analyte response in a biological sample that is spiked with the analyte and processed, with the response in a biological blank sample that is processed and then spiked with the analyte.
- Recovery of the analyte does not need to be 100%, but the extent of recovery of an analyte and of the IS (if used) should be consistent. Recovery experiments are recommended to be performed by comparing the analytical results for extracted samples at multiple concentrations, typically three concentrations (low, medium and high).

In line with Current FDA guideline.

Feedback from delegates

When using a stable label internal standard, recovery doens't have an impact if sensitivity of the method is sufficient. This investigation should be skipped if a stable label IS is used. If this investigation is not skipped, two concentration levels are fully sufficient. There is no reason why the mid level should behave differently from the highest level.

In my eyes, recovery does not necessarily be evaluated. A successful analysis of QC samples should be sufficient.

Recovery section is irrelevant for endogenous analytes as no absolute known concentration is available. Either need to be removed, or re-written to be specific to spiked additional amounts of analyte

EBF recommendation

Extraction recovery

- EBF does not consider extraction recovery to be a required assay validation parameter as absolute recovery is not critical. Consistency/reproducibility of recovery will be evident from pre-study validation and/or within study assay performance.

Tiered approach into practice: scientific validation for chromatography-based assays in early development – a recommendation from the European Bioanalysis Forum

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Acknowledgment and questions

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