New EMA guideline on method validation and how it translates into best practice for Ligand Binding Assays

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General comments on new EMA guideline

- EMA acknowledged that LBA differ significantly from chromatographic methods, therefore separate recommendations are provided → separate chapter 7
  - Reference standard
  - Specificity
  - Selectivity
  - Calibration curve
  - Precision and accuracy
  - Dilution linearity and parallelism

Reference standard

EMA

1. Well-characterized and documented, e.g. CoA and origin ✓
2. Strongly recommends to use same batch for C/QC prep as used for non-clinical and clinical studies ✓
3. Change of batch: analytical characterisation and bioanalytical evaluation required to ensure performance characteristics of method not altered ✓

→ Ad 2) in general, yes we do, at least for non-clinical studies, but often difficult for long clinical studies

→ Change of batch (including critical reagents):
Comparison of new reagent versus old: precision and accuracy run with 2 calibration curves and 2 full sets of 5 QCs on the same plate

Specificity

related to concept of cross-reactivity with structurally "related compounds"

„Endogenous compounds" → endogenous counterparts??

EMA

1. Timing: Evaluation may be conducted once „related molecules" are available (can be after original validation)
2. **Add increasing conc of the structurally related molecule or the drugs expected to be concomitantly administered at LLOQ and ULOQ**
3. Bias ± 25% from nominal value

→ In general, cross-reactivity / interference is tested during assay development to
→Select right tools and assay format

→ Different molar ratios of interfering protein to analyte must be tested
Bias ± 20% from nominal value

Selectivity

ability to measure the analyte in presence of unrelated compounds in matrix

EMA

1. ≥ spiking at least 10 sources of sample matrix at or near LLOQ
2. Should include **lipemic and haemolysed** samples
3. Strongly recommends to include sources from disease population
4. It may be prudent to evaluate selectivity at higher analyte conc
5. If interference is conc dependent → determine the *minimum* concentration where interference occurs. It may be necessary to adjust the lower level of quantification accordingly, before assay validation
6. Bias ± 20% (25% at the LLOQ) for at least 80% of the matrices

Ongoing discussion: to include also lipemic and haemolysed samples

How many? Degree of haemolysis? Flag sample

Calibration curve

Response function generally non linear and often sigmoidal

EMA

1. A minimum of 6 CS, at least in duplicate ✓
2. Spaced evenly on a logarithmic scale within anticipated range ✓
3. Anchor points allowed to facilitate curve fitting
4. Minimum of six runs during validation → results to be reported in a table to establish overall robustness of the regression model ✓
5. Bias ± 20% (± 25% at LLOQ and ULOQ) for at least 75 % of the CS. No acceptance criteria on anchor points ✓
6. CS may be prepared in surrogate matrix ✓

→ CS at LLOQ and ULOQ included → have to pass during precision-accuracy runs

Accuracy & precision

EMA

1. QC samples should be frozen
2. 5 QC samples at LLOQ, < 3 times LLOQ, mid, high, ULOQ
3. Assess accuracy, precision and **total error**
4. At least six independent runs over several days
5. Bias ± 20% (± 25% at LLOQ/ULOQ); Precision: < 20% (< 25% at LLOQ/ULOQ; **Total error: 30% (40% at LLOQ/ULOQ)**





→ Frozen QC:

- Which storage temperature?
- Prepared in bulk for all 6 runs? OR prepared for each run?
- Systematic error?

→ Total error: what is the added value?

Dilution linearity & parallelism

to check if conc of analyte > ULOQ can be accurately measured and to detect hook effect

EMA

1. QC samples > ULOQ to be diluted in blank matrix ✓
2. Back-calc conc: ± 20% of nominal conc
   Precision across all dilutions < 20% ✓
3. Parallelism between standard curve and serially diluted samples to be assessed to detect matrix effect and **differing affinities for metabolites**
4. Timing: as soon as study samples become available ✓
5. Study sample (close to Cmax) to be diluted to at least three conc with blank matrix ✓

➡ We allow also dilution in buffer if acceptance criteria are met.

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