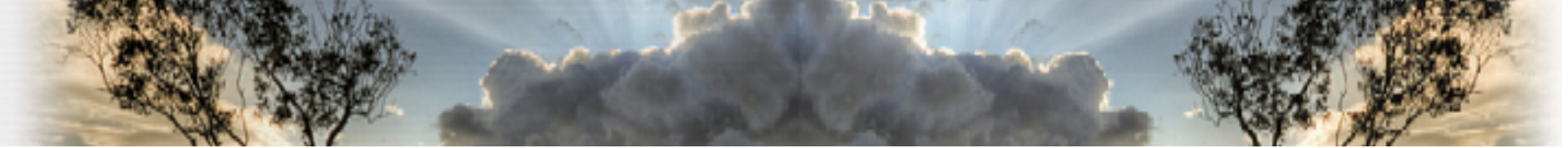

Considerations on Specificity and Selectivity for MS/MS assays

Timothy Sangster, on behalf of the EBF

Selectivity ICH M10

- 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 is 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 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 for each matrix.

Selectivity ICH M10

- Discussion points
  - Acceptance criteria – will 5/6 be acceptable.

- Suggested changes -

- Use of fewer sources may be acceptable in the case of rare matrices *and when scientifically justified for non-clinical matrices.*

Selectivity ICH M10

- 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, it is acceptable to spike matrix 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 preclinical 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 are obtained by spiking matrix with haemolysed whole blood (at least 2% V/V) **to generate a visibly detectable haemolysed sample**.

Selectivity ICH M10

- 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, it is acceptable to spike matrix 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 preclinical 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 are obtained by spiking matrix with haemolysed whole blood (at least 2% V/V) to generate a visibly detectable haemolysed sample.

Selectivity ICH M10

- Discussion points
  - Definition of ‘abnormally high levels of triglycerides’ to avoid any misunderstandings e.g. Triglyceride levels of > 300 mg/dL (3.4 mM/L)
  - The selectivity of lipaemic and haemolysed should be done as a for cause investigation based on method development.
  - Next slide – data to support.

Frequency of testing and **Failure** rate

Chrom

	Preclinical	Clinical
Hemolysed tests	288	307
Failed tests	4 (1%)	7 (2%)
Hyperlipidemic tests	192	271
Failed tests	0	3 (1%)

LBA

	Preclinical	Clinical
Hemolysed tests	100	127
Failed tests	1 (1%)	3 (2%)
Hyperlipidemic tests	17	118
Failed tests	1 (6%)	2 (2%)

Source : EBF Focus Workshop in collaboration with the AAPS and JBF: industry input in ICH M10, Lisbon, September 2017

Selectivity ICH M10

- 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, it is acceptable to spike matrix 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 preclinical 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 are obtained by spiking matrix with haemolysed whole blood (at least 2% V/V) to generate a visibly detectable haemolysed sample.

Selectivity ICH M10

- Discussion points
  - Definition of ‘abnormally high levels of triglycerides’ to avoid any misunderstandings e.g. Triglyceride levels of > 300 mg/dL (3.4 mM/L)
  - The selectivity of lipaemic and haemolysed should be done as a for cause investigation based on method development.
- Suggestion
  - ‘…to generate a visibly detectable haemolysed sample.’ This text adds no value and suggest it should be removed.

Specificity ICH M10

- 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, isomer, 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).

- Discussion points
  - Is the specificity or selectivity? Suggest combining into single section? Do we really care?

Specificity ICH M10

- 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**.

- Discussion points
  - Does the evaluation of Molecular weight and chromatographic separation remove the need for the quantitative assessment or are both expected?
  - Define ‘related substances…’

- Changes proposed - LC-MS should be changed to just MS to allow for GC-MS based methods.

Ref: Co-medication and interference testing in bioanalysis: a European Bioanalysis Forum recommendation BioAnalysis Vol.8 No.19

Specificity ICH M10

- 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.

- No issues.

Specificity ICH M10

- 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 (i.e., 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 discussed in the Bioanalytical Report.

- Discussion Points
  - Suggest this is moved to stability or Incurred Sample Stability?
- Suggested changes -
  - While acknowledging the difficulty for early stages it appears to still be expected – clarify.

Summary of Discussion Topics

- Selectivity
  - Acceptance criteria – will 5/6 be acceptable. Define acceptance more clearly what do we propose?
  - Definition of ‘abnormally high levels of triglycerides’ to avoid any misunderstandings e.g. Triglyceride levels of > 300 mg/dL (3.4 mM/L)
  - The selectivity of lipaemic and haemolysed should be done as a for cause investigation based on method development
- Specificity
  - Is the specificity or selectivity? Suggest combining into single section? Do we really care?
- Specificity – Back Conversion
  - Suggest this is moved to stability or Incurred Sample Stability? Thoughts?

Suggested comment to EMA/EWG

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

Acknowledgment and questions

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