The essence of Matrix effects for chromatographic assays

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Focus Workshop

(*In collaboration with the AAPS and JBF*)

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Problem statement / Introduction

- Matrix can affect ionization of the analyte(s)
- This matrix effect can therefore impact data accuracy and precision
- *The influence of matrix effects on the accuracy and precision needs to be evaluated and controlled*

Matrix effect evaluation

- Qualitative: *Post Column Infusion (Enhancement or Suppression in the signal).*

- Quantitative: $MF = \frac{peak\ area\ with\ matrix}{peak\ area\ without\ matrix}$

Guidelines on matrix effect for LC-MS

(FDA draft guidance)

- *…..Appropriate steps should be taken to ensure the lack of matrix effects throughout the application of the method, especially if the matrix used for production batches is different from the matrix used during method validation. Matrix effects on ion suppression or enhancement or on extraction efficiency should be addressed. …..*

(EMA guideline)

- *For each analyte and the IS, the matrix factor (MF) should be calculated for each lot of matrix, by calculating the ratio of the peak area in the presence of matrix (measured by analysing blank matrix spiked after extraction with analyte), to the peak area in absence of matrix (pure solution of the analyte). The IS normalised MF should also be calculated by dividing the MF of the analyte by the MF of the IS. The CV of the IS-normalised MF calculated from the 6 lots of matrix should not be greater than 15 %. This determination should be done at a low and at a high level of concentration (maximum of 3 times the LLOQ and close to the ULOQ).*

Guidelines on matrix effect for LC-MS

(MHLW guideline)

- *Matrix effect is an alteration of the analyte response due to matrix component(s) in the sample. Matrix effect should be assessed when using mass spectrometric methods. Matrix effect is evaluated by calculating the matrix factor (MF). The MF is determined by comparing the analyte response in the presence of matrix with that in the absence of matrix. MF should be calculated using matrix from at least 6 different sources. The MF may be normalized by its internal standard. The precision of the MF calculated should not exceed 15%. Matrix effect can also be evaluated by analyzing QC samples, each prepared using matrix from at least 6 different sources. The precision of determined concentrations should not be greater than 15%.*

(Anvisa Resolution)

- Very similar to EMA

Guidelines on matrix effect for LBA

(**FDA draft guidance**): *The calibration curve in biological fluids should be compared with calibrators in buffer to detect matrix effects using at least ten sources of blank matrix.*

(**EMA guidelien**): *Selectivity is tested by spiking at least 10 sources of sample matrix at or near the LLOQ. These sources should include lipemic and haemolysed samples. It is also strongly recommended that sources from relevant disease population be included. Selectivity should be evaluated at the low end of an assay where problems occur in most cases. It may be prudent also to evaluate selectivity at higher analyte concentrations.*

(MHLW guidance): *Selectivity is evaluated using blank samples obtained from at least 10 individual sources and near-LLOQ QC samples (i.e., QC samples at or near the LLOQ) prepared using the individual blank samples. In the case of a matrix with limited availability, it may be acceptable to use matrix samples obtained from less than 10 sources.*

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Current challenges

- Difference between EMA, FDA, ANVISA, MHLW
- Difference between LBA and LC-MS
- Matrix factor is an artificial / academic measure of matrix effect and may not be a good reflection of experimental conditions
- Matrix factor determination is prone to error

Can we come up with an alternative way to evaluate matrix effects in an LC/MS bioanalytical method?

Can we come up with similarity between LC-MS and LBA to evaluate matrix effects? QC-type approach? This would be in compliance with FDA and MHLW. Could easily be assesses together with selectivity.

EBF survey

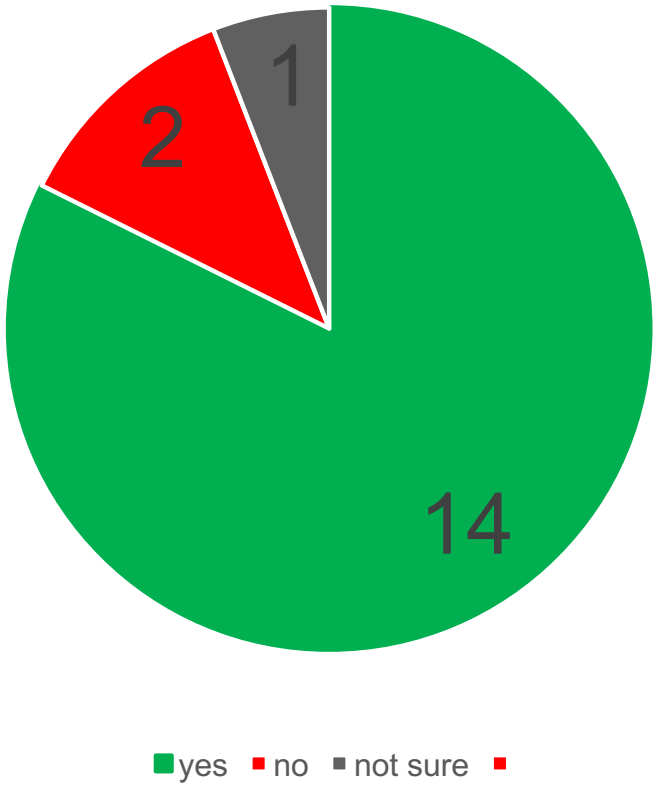
- Would you / your company be in support proposing following ‘best practice’ as a suggestion for the ICH M10 guideline in relation to spiked QC experiment (instead of matrix factor determination) to evaluate matrix effects ?

- If yes, how many QC levels would you like to evaluate:
  - Only mid-QC
  - Only low- and high-QC?
  - Low-, mid- and high-QC?

- Comments?

EBF survey responses (17)

QC's to evaluate matrix effect?



12 yes-votes for low and high-QC
2 yes-votes for mid-QC only

EBF survey comments

- The MF is a good indicator of how your assay is performing and in some case it might even be worthwhile to examine MF in multiple column lots (with example why)
- Suggestion to frame it like the LBA world: spike at low level":

7.1.1.3. ~~Selectivity~~ Matrix effect/individual recovery

~~Selectivity~~ Matrix effect/individual recovery is tested by spiking at least 10 sources of sample matrix at or near the LLOQ. ~~These sources should include lipemic and haemolysed samples~~. It is also strongly recommended that sources from relevant disease population be included. Matrix effect/individual recovery should be evaluated at the low end of an assay where problems occur in most cases. It may be prudent also to evaluate selectivity at higher analyte concentrations.

- Similar comments from LBA experts
- *"We have not 100% agreement on this here but the majority says "yes", which indicates that this is a major change for some to cope with"*

Summary of EBF survey

- Majority of (EBF) community is open to simplify evaluation of matrix effect
- Majority of (EBF) community in favor of eliminating matrix factor determination as part of validation package
- This change is probably consider to be “major” and requires broader discussion and consensus

(draft) Recommendations Matrix Effect

- Matrix effect should be evaluated using a QC-type experiment.
- QC's to be prepared in at least 6 different matrices + …
- Precision should be ≤ 15%

- For discussion:
  - Do we need criteria for accuracy?
  - Should haemolysed / hyperlipidemic matrices be included?
  - Disease state matrices?
  - QC high / QC low ?
  - How many replicates?

Acknowledgement

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