Workshop on ICH M10

PC-04 - Carry over assessment during samples analysis

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14 November 2023 – Barcelona, Spain

From the guideline

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.

Pre-meeting survey

	the question	Yes	No
Q1	Are you clear on the requirements for carry-over assessment and reporting during samples analysis?	20	3
Q2	If not, what are the ambiguities you see?		
Q3	How do you evaluate the detected carry over impact on the measured concentrations during sample analysis after ICH M10?		
Q4	How do you mitigate detected in the analysis carry over?		
free text			

Key message from the pre-meeting survey comments

- ICH M10 requirements for carry-over assessment and reporting during sample analysis appear clear;
- Although, there is a good agreement on the approaches for mitigation of carry over during samples analysis, still there are many different interpretations on how to evaluate it;
- Best practices and recommendations are summarized by synthesizing the most widely applied approaches.

Q3	How do you evaluate the detected carry over impact on the measured concentrations during sample analysis after ICH M10?

Recommended “best or common” practice from responses:

- Evaluate signal in control blank samples after high concentration:
  - after ULOQ
  - after high QC

Q4	How do you mitigate detected in the analysis carry over?

Recommended “best or common” practice from responses

- Reduce analytical range;
- Do not randomize samples, if possible (i.e. run in profile order);
- Inclusion of additional blank/solvent injection.

Raw data from the pre-meeting survey comments

- In the next slides we provide the unredacted details from 56 survey files reaching us prior to the deadline.
- Surveys that have arrived after the deadline could not be included anymore, for logistic reasons. Please speak up if your comment wasn't already captured in the other 56 files

Q1: Are you clear on the requirements for carry-over assessment and reporting during samples analysis?

- Yes
- Yes - If indicated by Validation set more blank in the sample analysis run
- Yes, carry over should not be more than 20%.
- Yes - M10 state use the Highest Calibrator (ULOQ) - double blank - LLOQ

Q2: If not, what are the ambiguities you see?

- Are alternative approaches acceptable
- None, I believe this can be left open for scientific judgement
- no recommendations for carryover assessment during sample analysis
- If <20% of LLOQ is allowed for all samples, non-randomisation does not help. What % is acceptable if carry-over is unavoidable?
- "The wording ' During validation carry-over should be assessed by analysing blank samples after the calibration standard at the ULOQ'. The wording 'samples' is difficult to understand but common practise in to use 'double blank'. "

Q3: How do you evaluate the detected carry over impact on the measured concentrations during sample analysis after ICH M10?

compare signal in blank (after highest cal) to LLOQ	20% of Cal 1 area	Driven by SOP with mitigation based on carry-over and sample concentration	Only, if blank sample after ULOQ calibrator is >20% of LLOQ calibrators: Carry-over from ULOQ calibrator into the blank is set to 100%, each individual peak area is then investigated regarding the theoretical increase by the previous sample	Evaluate blank samples after High concentration > 20% LLOQ

in-study monitoring of carryover during sample analysis	If CO is greater than the 20% highlighted we would typically fail the batch, resolve the issue returning the system back to baseline 'cleanliness' and reanalyse.	yes but we already did that	same as previously	We already applied the carry-over assessments calculation

Q3: How do you evaluate the detected carry over impact on the measured concentrations during sample analysis after ICH M10?

calculate impact on individual samples	only when applicable, the carryover of the previous sample will be calculated for each sample	Mitigation blanks after ULOQ samples at the beginning and end of each run	I do not do carry over	If you have carryover develop more...do not validate or cut the range

If you have carry over and you did not before maintain equipment better.	blanks after high concentration	Samples at the LLOQ (n = 12) and ULOQ level (n = 6) should be analysed against a calibration curve. The order of sample injection on the autosampler should be as follows: Calibration Curve, ULOQ, LLOQ-1, LLOQ-2, ULOQ, LLOQ-1, LLOQ-2, etc. (with LLOQ-1 first replicate of LLOQ sample and LLOQ-2 second replicate of LLOQ sample) OR, double blank samples and ULOQ level should be analysed against a calibration curve. In this case, at each analytical run, the order of sample injection on the autosampler should be as follows: double blank, Calibration Curve, double blank-1. This study may also be performed as part of the assay pre-validation. If it is conducted in pre-validation, documentation should be included as an appendix to the validation report.

Q3: How do you evaluate the detected carry over impact on the measured concentrations during sample analysis after ICH M10?

Calculate if the effect of the carry-over on each sample is greater than the variability of the method	if no alert during method validation : no specific evaluation	the batch is not considered if carry over, we fix the problem and reinject with no carryover	carryover (Blank Sample) injection after the ULOQ calibration standard	Double blank samples after each ULOQ and High QC samples

Analytical method is not accepted unless carry over absence is confirmed during development and validation	Injection depending on concentrations (Low concentration to high concentration) or addition of control blank after high sample values	Specificity criteria. If not met Evaluation involving the actual results (sample concentrations and concentration profiles, pre-dose samples/blank and zero samples/calibration standards and QC samples) and the measurement sequence of the samples.	Injecting blank sample after higher calibration standard during and before batch. The process was already there in practice before ICHM10	With blanks injected after ULOQ and after each high QC concentration.

Q3: How do you evaluate the detected carry over impact on the measured concentrations during sample analysis after ICH M10?

Carry over is assessed in the system suitability assessments and in the validations.	In each run containing a calibration curve, 3 zero serum samples are analyzed directly following the sample at the highest calibration level and all high level QC samples. Response of the analyte in all samples has to be <20.0% from the response (peak area) found in the sample at the LLOQ level analyzed in the same run	the batch is not considered if carry over, we fix the problem and reinject with no carryover
If there is an unexpected carryover , samples after an high concentration and which could be impacted are reanalyzed	Evaluation is done using peak area value and not concentration values. M10 also specify to use 'analyte response'.	carryover blanks

Q4: How do you mitigate detected in the analysis carry over?

study director evaluates impact under consideration of any other measured samples in this run (Cal curve, other blanks, pre dose)	Change of wash solvent, additional blanks after high samples, if possible	Driven by SOP with mitigation based on carry-over and sample concentration	avoid randomization of samples	More blanks; non-randomised sample analysis
blanks, solvent samples, no randomization, profile order	Narrow assay ranges where need be or reanalyse when carryover has been resolved.	The potentialy impacted samples are re-scheduled as analytical repeats.	run samples in profile where possible and control it with carryover blanks	preventive measures like additional blanks; sequence of analysis if possible

Q4: How do you mitigate detected in the analysis carry over?

no randomization - analyze samples based on PK profile	Can add blanks now too if needed	during development we set tigher criteria on carryover.	inject injection sovent(s) between each sample	most of time by adding eluant between each injection

Non-randomisation of study samples, injection of blank samples after samples with an expected high concentration	e.g. by adjustment of range, blank injections, analyze by profile	In all cases: if unknown conc is > to the ULOQ, the following sample is reassayed In case of carryover is observed during method validation: injection of samples in reverse order (IV route) and additional blanks after ULOQ	Evaluate the impact of the carry-over on the sample concentration or raise the LLQ to CAL2.

Q4: How do you mitigate detected in the analysis carry over?

if there is carryover, we fix the issue and re-inject.	Washing solution, washing time, mobile phase	Injection of blank samples	By injecting blank matrix extract / reagent blank sample(Reconstitution solution) after each high concentration samples in the batch

If the carry over cant be eliminated in the R&D phase or method adjustments, we will add a double blank after the highest concentrations. In sample analysis run, subject samples and QC as disperesed so that carry over is mitigated.	if there is carryover, we fix the issue and re-inject.	Avoiding sample randomisation, injection of blank samples after samples with an expected high conc.	Implement in the method SOP

randomizing samples in run and additional blanks/ extended wash time/volume	we make sure to not have any carryover.