Which steps to take if re-analysis results do not confirm the result of the first analysis?

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Introduction

- Toxicity study by oral gavage administration to beagle dogs for 13 weeks (GLP)
- In total 1400 plasma samples to be analyzed
- Analyses were done using a validated LC-MS/MS assay
- Stability was proven in plasma and blood

First analysis and Re-analysis

- As high plasma concentrations were expected, study samples were analyzed after 2-, 5-,10, 20- or 50-fold dilution
- 159 samples had to be re-analyzed because of the following reasons:
  - Above quantification limit (AQL): 146 samples
  - Below quantification limit (BQL): 11 (diluted) samples
  - Internal standard criterion not met: 2 samples
- Re-analysis was not confirmed for:
  - AQL samples: 1 sample
  - BQL samples: 11 samples (all!)

First analysis and Re-analysis

- 11 overdiluted samples were re-analyzed undiluted

First result (BQLs) (ng / mL)	Expected result (ng / mL)	Second result (ng / mL)
<40.0	2 – 40	133
<40.0	2 – 40	456
< 100	2 - 100	671
< 100	2 - 100	522
< 100	2 - 100	952
< 100	2 - 100	778
< 100	2 - 100	1500
< 100	2 - 100	1440
< 100	2 - 100	582
< 100	2 - 100	380
< 100	2 - 100	823

⇒ How did we proceed?

Use of ISR approach in failed 'Re-analysis' investigation

- Formal ISR experiment was not performed for this study
- An experiment, in which the principles of ISR were applied, was performed to document potential reasons of assay failing
- 10% (140 samples) were re-analyzed
  - Difference (%) was calculated as follows:

    $((\text{repeat} - \text{original})/\text{original}) \times 100 \leq 20.0\%$
  - The same dilution factors were applied
- The performance of an ISR experiment was described in an amendment to the protocol

Results

- Criterion was not met for 102 of 140 samples (72.9%)
- High % difference could be observed for undiluted as well as for diluted samples
- % bias ranged from -84% to +489.9%
- Majority of failed re-analyses originated from diluted samples
- Re-analysis failure was independent of the dilution factor

- Reproducibility of the data was not proven and reliability of the original results could not be guaranteed.
- Results of first analysis were rejected

- In order to assess the failure in more depth, failed re-analysis investigation was continued.

Failed re-analysis investigation - continued

- Swap of plasma samples was checked:
  - Labels on the tubes
  - Sequence of the study samples in the racks
  - Sequence of the vials
- Parameters in method validation were checked:
  - Validation of 10- and 100-fold dilution QCs
  - Long-term stability data: 414 days were proven
  - previous ISR experiment: passed the acceptance criterion
  - Considering the size of the study and the number of study samples that had to be diluted, using 5 different dilution factors, dilution or human error could have occurred.

How would you solve this problem to fulfill the scientifically and compliance demands?

Janssen's approach: re-analysis in duplicate

- Actions taken:
  - All samples were re-analyzed in duplicate (n = 2800)
  - The available LC-MS/MS assay was used for the re-analyses
  - Special attention was given to the preparation of the dilution of the samples:
    - Only 10-fold and 50-fold dilutions were used
  - Each sample was re-analyzed in duplicate over two different runs
  - Analyses were done by two analists, working independently from each other
  - Acceptance criterion: difference between both results ≤30%

    $(X-Y)/(X+Y) \times 200 \leq 30\%$
  - If the %difference was ≤30%, the first result was reported
  - If the %difference was >30%, no result was reported

Results of re-analysis in duplicate

- 99% of the samples met the re-analysis criterion (only 3 samples did not fulfill the re-analysis criterion)
- Considering the robustness of the method, the special attention given to the preparation of the dilution of the study samples and the % samples that met the re-analysis criterion (99%), it can be concluded that the results of the re-analyses are reliable.
- The failed run investigations and the results of the ISR experiment and re-analyses in duplicate were extensively described in the bioanalytical report.

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