cBARS / PK Sciences

Acceptance criteria for method validation and sample analyses of a protein by LC-MS/MS

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NOVARTIS

Analyte and methods in the discussion

- **Type of molecule**: Humanized monoclonal antibody
- **Purpose of assay**: PK determination in clinical studies
- **Method**: LC-MS/MS after pellet digestion (trypsin)
- **Internal standard**: stable isotope labelled-whole protein
- **Quantification**: Specific signature peptide (1263 Dalton)
- **Dynamic range**: 0.25-250 µg/mL
- **Project period**: 2014 - Present

Acceptance criteria used

- ± 20%, 25%@LLOQ [20/25%], similar to LBA criteria
- No clear guidance/guideline for protein quantification by LC-MS/MS at that time
- Refer to the White papers -> Novartis SOP

  Recommendations for validation of LC-MS/MS bioanalytical methods for protein biotherapeutics. The AAPS Journal, 17 (1), 2015

Table I. Comparison of Conventional Method Validation Parameters for Protein LBA and Small Molecule LC-MS/MS, with those Proposed for Protein LC-MS/MS

Parameter	Protein LBA	Small molecule LC-MS/MS	Protein LC-MS/MS, using a surrogate peptide (recommended)
Calibration curve regression function	Non-linear with 4 or 5 parameter logistic. Anchor points may be used	Linear preferred, non-linear with justification	Linear recommended when possible; non-linear models may be acceptable with some affinity capture methods
Lower limit of quantification (RE, CV)	Within ±25%	Within ±20%	Within ±25%
Calibration standards (RE, CV)	Within 20% (except LLOQ and ULOQ)	Within 15% (except LLOQ)	Within 20% (except LLOQ)
Accuracy and precision (RE, CV)	Within 20% (LLOQ/ULOQ QCs within 25%). Min. 6 runs	Within 15% (LLOQ QC within 20%). Min. 3 runs	Within 20% (LLOQ QC within 25%). Min. 3 runs
Dilutional integrity/linearity	RE, CV within 20%	RE, CV within 15%	RE, CV within 20%
Parallelism	Dilution series CV within 30% using incurred samples	NA	NA; may be used for troubleshooting affinity capture methods

To be published later

Conclusion

Experimental data comparison between original (20/25%, some are rejected by 15%) and reanalyzed values (15/20%)

- No relevant difference between the two datasets.

Simulation of two assays 15/20% (BE, better) and 20/25% (EBE, good)

- No relevant difference in PK parameters.

By the way....

- Stability assessment
  - Need to extend the LTS up to 39 months (being extended)
- Time
  - From decision to completion of reanalysis:
    # half year with lots of discussion,
    # contract,
    # additional sample shipment,
    # reprocessing the data and
    # sample reanalysis
- Cost
  - Reprocessing,
    backup sample shipments,
    ca 350 sample reanalysis over 10 analytical runs,
    additional repeat of method validation items

Question to Audience

We use LC-MS/MS for protein quantification in this case. If we did not, we used LBA.

- **Since LBA uses 20/25% acceptance criteria, Why we need to use 15/20% criteria for LC-MS/MS determination for protein quantification?**
- **Do your Pharmacokineticist / statistician interpret the dataset with 15/20% (chromatography) and that with 20/25% (LBA) different way?**
- **How much the narrowed criteria by chromatography assay contributes to improvement to the entire results?**

Question to Audience –continued–

- Can we (EBF) discuss the acceptance criteria of protein quantification with relevant stakeholders?
- Do we need to have two different acceptance criteria, 15/20% (Chromatography) and 20/25% (LBA) for protein quantification?
- If we harmonize the criteria, which one is appropriate?
- Any consideration by type of the study,
  e.g. BioE, high risk drug link to the exposure?

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Q & A