The Regulatory Space – Introduction to session

Presenter: Magnus Knutsson on behalf of EBF

Bioanalytical Strategies for Large Molecules in Modern Drug Development: LBA and LC-MS united

21 June 2017

Lisbon, Portugal

Method validation: acceptance criteria

- Is 'Size of molecule' or 'Technology' the driver to define acceptance criteria?
  - Technology as driver: "its LC-MS/MS so LC-MS/MS rules apply"
    - Do we go back to pre-CCII criteria, e.g. because potential lack of Stable Isotope internal standards (resulting in pre-CC-II quality for MS/MS)?
    - What about hybrid methods, e.g. immunoaffinity sample prep combined with LC-MS/MS
  - Size of molecule as driver: "it's a large molecule, so LBA rules apply"
    - What is a Large Molecule?

Method validation: acceptance criteria

- Evolving science, we are still learning
- None or very little guidance in current guideline/guidance
- Limited experiences also by the regulators

Some guidance in Industry papers

Reflection on criteria

Bioanalysis. 2013 Sep;5(18):2211-4. doi: 10.4155/bio.13.193.

LC-MS/MS of large molecules in a regulated bioanalytical environment - which acceptance criteria to apply?

Knutsson M[1], Schmidt R, Timmerman P.

Discussing the science

AAPS J. 2015 Jan; 17(1): 1–16.
Published online 2014 Nov 13. doi: 10.1208/s12248-014-9685-5
PMCID: PMC4287296

Recommendations for Validation of LC-MS/MS Bioanalytical Methods for Protein Biotherapeutics

Rand Jenkins, Jeffrey X. Duggan, Anne-Françoise Aubry, Jianing Zeng, Jean W. Lee, Laura Cojocaru, Dawn Dufield, Fabio Garofolo, Surinder Kaur, Gary A. Schultz, Keyang Xu, Ziping Yang, John Yu, Yan J. Zhang, and Faye Vazvaei

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"The current thinking from the EBF Topic Team is to start with a conservative approach when defining acceptance criteria and not to propose acceptance criteria that are still too demanding for the technology/analytical approach..."

- As always, there might be situations where alternative acceptance criteria can be defendable to achieve valid results for a given analytical challenge without jeopardising the safety of the patient
- Establish acceptance criteria a prior to start of method validation, based on method establishment experience

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LC-MS/MS of large molecules in a regulated bioanalytical environment - which acceptance criteria to apply?

Knutsson M[1], Schmidt R, Timmerman P.

- General recommendation from the paper
- Smaller intact analyte assay (e.g. peptides and oligonucleotides)
  - 4-6-15
- Larger intact analyte assay (especially hybrid approaches)
  - 4-6-20
- Digest analyte assay
  - In most cases, 4–6–20 acceptance criteria will be achievable
  - Taking the increased complexity of these assays into consideration there might be situations and assays where a widening of the acceptance criteria beyond 4–6–20 based on method establishment data should be allowed.

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- Recommendation on Protein LC-MS/MS by Surrogate peptide approach

Validation Parameters

Parameter	Protein LBA	Small Molecule LC-MS/MS	Protein LC-MS/MS via Surrogate Peptide (Recommendation)
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; non-linear models may be acceptable with some affinity-capture methods
Lower Limit of Quantification (RE, CV)	25%	20%	25%
Calibration standards (RE, CV)	20% (except LLOQ and ULOQ)	15% (except LLOQ)	20% (except LLOQ)
Accuracy & 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

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- Some technical concerns
  - Reference Standard
  - Enrichment
  - Digestion
  - Recovery
  - Monitoring peptides
  - Internal Standards
  - Affinity capture considerations