15th Open Symposium

The Bioanalytical Compass

Navigating to our True North

Past, current and future of EBF discussions, challenges and recommendations on Protein MS

Matt Barfield, on behalf of the EBF

16-18 November 2022, Barcelona

The WHY?

The challenges haven't really changed over the last 10 years

To understand this statement we need to understand the history

- To give an overview of where the EBF has influenced, discussed and help drive the conversation on protein analysis by LC/MS/MS since 2011
- Understand where we are today as a community and what EBF has planned


IN THE
BEGINNING

In the beginning: 2011

- **June 2011 Focus Meeting – Large Meets Small**
  - Connecting strategies on analysing large molecules with small molecule technologies
  - Bringing bioanalysis together (experts in LBA and MS from industry & academia)
  - Looked at technology developments, validation requirements, cutting edge approaches and the challenges including regulations

10 years on and we are still debating…….

Bioanalysis (2012) 4(6) 627-631

Method validation: acceptance criteria

- Do we have enough experience to judge?
  - Limited experience available to make a clear statement
  - A (potential) desire from the small molecule community to call LC-MS/MS of peptides/proteins ‘the same’ as LC-MS/MS of small molecules. But is this fair?
- Who still remembers the origin of 4-6-15(20) or 4-6-20(25) and, more importantly, the rationale?
  - Not that we want to challenge, but was 4-6-20(25) for chromatographic assays not good enough to document PK, safety and efficacy?
  - What drove/drives the difference in acceptance criteria for LBA vs. Chromatography?

Points of attention - Regulations

EBF Teams Formed 2012
- Strategy
- Regulations

Method validation: acceptance criteria

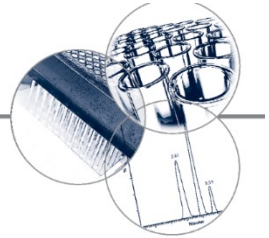
– Is ‘Size of molecule’ or ‘Technology’ the driver to define acceptance criteria?

  o 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 IS (resulting in pre-CC-II quality for MS/MS)?
    - What about ‘mixed technology methods’ (e.g. LBA sample prep combined with MS/MS detection?)

  o Size of molecule as driver: “it’s a large molecule, so LBA rules apply”
    - Can somebody give the definition of a Large Molecule?

Fast forward to 2014

EDITORIAL



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

(*Bioanalysis, 2013, Vol. 5, No. 18, Pages 2211-2214*)

Changing the technology should not trigger changing the acceptance criteria if there is no compelling safety or PK need

Conclusion:

The EBF is pleased with the increased possibilities offered by LC–MS(/MS) to the bioanalytical scientist for the analysis of peptides and proteins. As part of their current ongoing discussions, it is the EBF's current thinking not to copy regulated requirements for small-molecule bioanalysis for peptides and proteins when analyzing them using LC–MS(/MS), with the exception of small intact peptides. At the same time, we want to focus the scientists' attention on the potential complementary information generated by LC–MS in addition to LBA data on a specific large molecule as an important strategic opportunity to increase the PK/PD knowledge. Hence, the use of both technologies should be considered and LC–MS should not necessarily replace LBA for peptides and proteins.

The story continues 2017

- Focus workshop: Bioanalytical Strategies for Large Molecules in Modern Drug Development: LBA and LC-MS United
- Focusing on
  - What do we need to measure?
  - **What are we measuring? How does the technology impact the results?**
  - **The regulatory space**
  - learning your molecule
  - developing your molecule

EBF - Focus Workshop

21-22 June 2017, Lisbon

Bioanalytical Strategies for Large Molecules in Modern Drug Development:

LBA and LC-MS United

The Regulatory Space – Acceptance Criteria

….are we afraid to ask the real questions?

Why, for the last 15+ years, are we accepting different acceptance criteria for LBA vs. CHROM assays, when we are making the same PK, PD, TK claims?

Was/is ‘4-6-20’ not good enough for all data? LBA or CHROM?

Is there value of even removing the label “CHROM” and “LBA” and refer to “PK assay” with 1 harmonized set of criteria → PK ASSAY

Has technology developments not allowed progressing to harmonize acceptance criteria for PK assays?

And, no

- This is no suggestion to bring LBA to 4-6-15
- But…a suggestion for the industry and regulators to reconsider 4-6-15 for chromatography and harmonize acceptance criteria for PK assays to the quality level which is sufficient to make valid decisions.
- It will remove the need for a non-added value discussion on defining ‘hybrid assay criteria’ or stimulating the industry to claim that an LC-MS/MS assay is actually an LBA assay in disguise.

Pharmaceutical Research, Vol. 11, No. 4, 1994

An Assessment of the 4-6-20 Rule for Acceptance of Analytical Runs in Bioavailability, Bioequivalence, and Pharmacokinetic Studies

Robert O. Kringle[1]

Received May 28, 1993; accepted September 30, 1993

A recent conference report described a decision rule, hereafter referred to as the 4-6-20 rule, for acceptance/rejection of analytical runs in bioavailability, bioequivalence, and pharmacokinetic studies. This procedure requires that quality control specimens at three concentrations (low, medium, and high) be assayed in duplicate in each run. For run acceptance, at least four of the six assay values must be within ±20% of their respective nominal concentrations, and at least one of the two values at each concentration must be within these limits. An inherent flaw in this decision rule is that the risk of rejecting runs, when the assay performance has in fact not deteriorated, varies for each assay and is neither known nor controlled. In this paper simulation methods are used to evaluate the operating characteristics of the 4-6-20 rule in comparison to those of classical statistical quality control procedures.

KEY WORDS: quality control; Shewhart control; multivariate control; operating characteristics; power.

Defining the acceptance criteria: Will 4-6-20 not be able to do the job?

- …knowing it did the job for at least a decade
- …and it still does for LBA assays,
- …and it was changed to 4-6-15 for CHROM with little or no consensus/scientific rationale?

The Next Installment 2018

- Publication: Towards decision-based acceptance criteria for Bioanalytical Method Validation: a proposal for discussion from the EBF
- 3 points were raised
- A challenge to the industry to have open discussions on whether it makes sense to move away from technology-based acceptance criteria in favor of decision-based acceptance criteria
- Reference: Bioanalysis (2018) 10(16), 1255-1259

Three Discussion Points

- **PK acceptance criteria**
  - Redefining acceptance criteria for Bioanalytical Method Validation and basing them on the decisions taken on the data – move away from technology based criteria

- Harmonized decision-based acceptance criteria can provide an acceptable answer to one of the key questions ‘Which criteria to use in so-called ‘hybrid assays’ (protein LC/MS/MS)

- Also answers current and future questions on acceptance criteria for new technologies where the end point is PK/safety

14

2020 – Re-Ignite

- Creation of a new focus group
- Protein analysis by LC/MS/MS
- Continue the journey and keep the discussion ongoing. The discussion's don't just impact Protein LC/MS/MS but all new future technologies that support PK/safety
- We need to have a simple solution, fit for the future

Name	Company
Amanda Wilson	Astra Zeneca
Mark Jean Gnoth	Bayer
Benno Ingelse	Byondis
Iain Love	CRL
Nico van de Merbel	ICON
Peter Blattmann	Idorsia
Fabrizia Fusetti	Genmab
Gregor Jordan	F. Hoffmann-La Roche
Rob Wheller	LGC
Sune Hove Sporring	Novo Nordisk
Mike Blackburn	Quotient Sciences
Stephane Muccio	Sanofi
Matt Barfield	F. Hoffmann-La Roche

Today's team

Current industry experience with measuring large molecule by LC-MS - A finger on the pulse survey outcome

- 2/3 companies that responded are using LC-MS for protein analysis
- Protein analysis by LC-MS is used across all phases of R&D
- The most used application is for PK assays
- Customers and stakeholder are not commonly involved in assay choice or acceptance criteria decisions
- It's a close call as to whether companies set acceptance criteria based on technology or by the scientific question being asked of the data
- The use of different acceptance criteria or technology in the measurement of the same molecule is rare
- Overall 4:6:15 criteria are applied however if immunoaffinity or digestion are applied then 4:6:20 is applied

Questions to Consider when Building a Bioanalytical Strategy for Proteins

- Sensitivity and Selectivity remain main drivers when selecting a bioanalytical platform though practical considerations also key
- Data generated on two platforms are both “true” – An understanding of why results are different is more important than any numerical difference. How is the communicated?
- Acceptance criteria for LC-MS assays of proteins: “it depends” – Depends on what? Can we standardise?

Ghent 2022

Goal of group:

Collate community expertise and provide influence to stakeholders on why, when and how to apply mass spec for (quantitative) protein analysis

Opportunities to provide publication, recommendation, further discussion for a
(focus workshops), decision making flow-chart…

… what does the community want from EBF in this space?

Publication strategy – the 2nd

- What needs to be measured?
- Technology selection
- Enzymes
- Pulldown
- Signature peptide selection
- Reference materials
- Automation
- Internal standards
- Pre-validation experiments

Publication strategy – the 1st

- Is Protein MS and specifically Hybrid MS
  - A LBA assay with a different detector?
  - A mass spec assay with a different sample prep?
  - A combination of LBA & MS with the pros and cons of each technique?
  - Going forward, it may be even less straightforward → into new territory
- ICHM10 – no mention or guidance. What about acceptance criteria?
  - ICH M10 was of course all about harmonisation and there was nothing to harmonise on hybrid assays → opportunity for industry to lead based on science
- The honest discussion – why do we as Bioanalysts only share the good?
- Regulatory fear – Why do we as Bioanalysts often assume the worst and default to belts and braces

A invitation to you all

An invitation to contribute to a focus workshop or cyber meeting to further discuss and continue EBF's leadership in this critical area

The door is still open and there is a blue galleon in view

Acknowledgements

To the many who have contributed over the years and those that continue to learn and challenge

Contact Information

Questions: info@e-b-f.eu

European Bioanalysis Forum vzw
www.e-b-f.eu