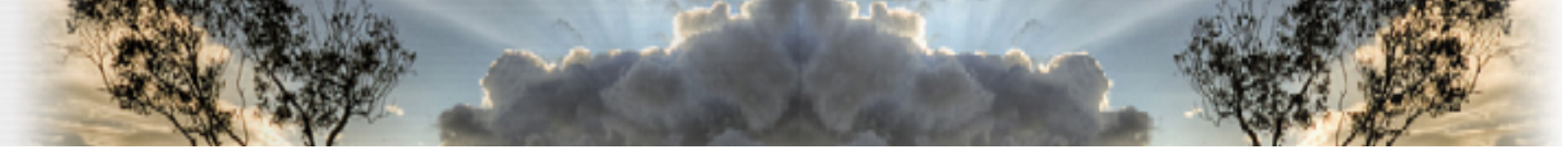


EBF

EBF Open Symposium

N° 13 From Cyberspace - Staying Connected

17-20 November 2020

Overview of EBF discussion and recommendations on protein analysis by LC-MS(MS)

Matt Barfield, on behalf of the EBF

Objectives of this presentation

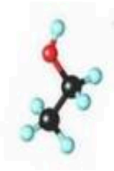
- To give an overview of where the EBF has influenced, discussed and help drive the conversation on protein analysis by LC/MS/MS since 2011
- Acceptance criteria – an EBF view
- What the future holds from an EBF perspective

In the beginning…

EBF 2011 Focus Meeting

Large Meets Small

Connecting strategies on analyzing large molecules with small molecule technologies



June 21-22 2011
Sheraton Brussels Hotel
Brussels, Belgium

– Brought bioanalysis together (experts in LBA and MS from industry & academia) around this theme for the first time

– Looked at technology developments, validation requirements, cutting edge approaches and the challenges including regulations

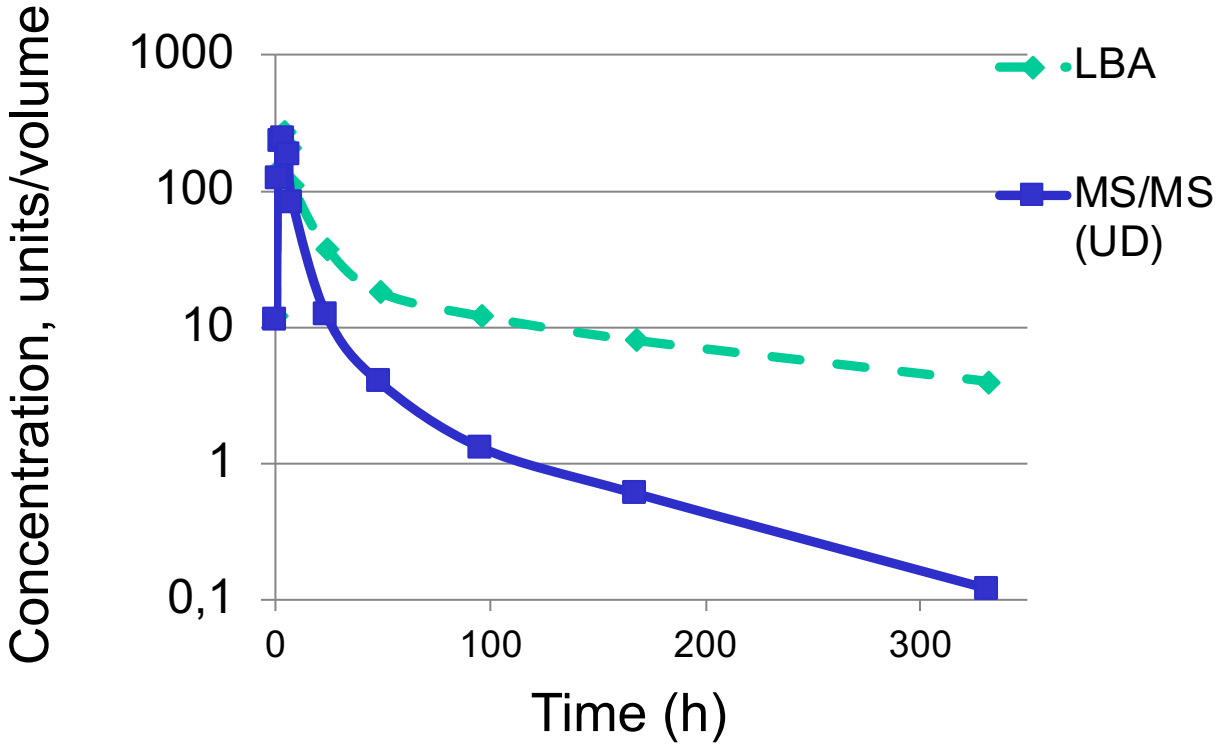
Bioanalysis (2012) 4(6) 627-631

CONFERENCE REPORT | NEWS & ANALYSIS

‘Large Meets Small’: connecting the bioanalytical community around peptide and protein bioanalysis with LC–MS(/MS)

– 9 years on and we are still debating…….

A simple example from the past
same drug X measured in serum with LC-MS/MS



Method comparisons / cross validations

- Cross validation aspects, do we anticipate:
  - 1-1 relationship between LC-MS/MS and LBA assay and why?
  - Differences between LC-MS/MS and LBA assay and why?
    - And how do we manage these differences from a PK, TK, PD perspective

- Scenario building of strategic use of LBA *vs.* MS/MS
  - Start with LBA and continue using LBA
    - Do we need to investigate specificity and selectivity better?
  - Start with LBA and switch to MS/MS
    - Extend the cross validation to reevaluation of PK/PD,…?
  - Start with MS/MS and remain on MS/MS
  - Start with MS/MS and switch to LBA
    - Extend the cross validation to reevaluation of PK/PD,…?

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

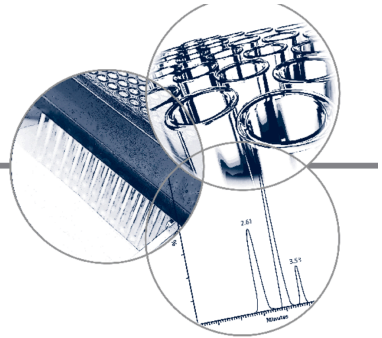
TTs Formed 2012 Strategy Regulations

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 ‘mixed technology methods’ (e.g. LBA sample prep combined with MS/MS detection?)
  - 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

- Inviting for continued discussion in a Editorial



EDITORIAL

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

"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…"

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

EBF

EDITORIAL

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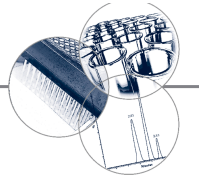

LC–MS/MS of large molecules in a regulated bioanalytical environment – which acceptance criteria to apply?

CRITERIA

VALUE

SYNERGY

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.

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

The story continues: 2017

EBF - Focus Workshop

21-22 June 2017, Lisbon

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

And again

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.

Additional reflections

- Do we have data to support our suggestion?
  - Has the difference between performance of LBA and Chromatography not become small enough to entertain the proposal?
  - Is emotion holding us back from taking a fresh look?

- The last decades, did we ever consider what the requirements for an assay needs to be?
  - Statistical power *vs.* BA criteria
  - Allowed bias *vs.* inter and intra subject biological variation

And biological variation can be bigger than the difference between 15 or 20 %

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 a decade, it still does for LBA assays, and it was changed to 4-6-15 for CHROM with little or no consensus/scientific rationale?

The Latest Installment 2018

A next invitation for discussion…

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Bioanalysis

Toward decision-based acceptance criteria for Bioanalytical Method Validation: a proposal for discussion from the European Bioanalysis Forum

Reference: Bioanalysis (2018) 10(16), 1255-1259

Three focal Points

- 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

Let's discuss

Conclusion & future perspective

With this manuscript, the EBF wants to propose an open discussion whether it makes sense to move away from technology-based acceptance criteria in favor of decision-based acceptance criteria. **We hope the discussion can get sufficient air time in industry, project teams and at upcoming meetings, either bioanalytically focused or with all stakeholders.**

We believe the proposal can alleviate the current ambiguity and nonadded value discussion on defining 'hybrid assay criteria'. Once integrated in our industry, harmonized decision-based acceptance criteria for bioanalytical assays in support of PK/safety will create a transparent platform to accept new technologies in the toolbox of the regulated bioanalytical (BA) scientist.

And last but not least, the proposal should be seen as refining the criteria for studies 'in scope' of the guidelines. As advocated during the AAPS/EBF/JBF sister meetings, criteria of studies 'out of scope' should not automatically be held to these criteria but should be driven by scientific rationale considering decisions taken from the assay data.

Let's discuss

Conclusion & future perspective

We hope the discussion can get sufficient air time in industry, project teams and at upcoming meetings, either bioanalytically focused or with all stakeholders.

And, still… No,

- …This is also not a proposal to bring acceptance criteria for chromatography-based assays to ±20% or for LBAs to ±15%. We are asking to define and agree on harmonized criteria, which can support the decision made on dosing, PK and safety from the bioanalytical data

- Input from the stakeholders about making these decisions is crucial

2020 – A new dawn

- Creation of a new project team in EBF
  - Protein analysis by LC/MS/MS

- Project goal
  - Continue the journey and help steering the discussion in what we feel is the need for industry.
  - The discussion's don't just impact Protein LC/MS/MS but all new future technologies that support PK/safety
  - Connect : Bring Industry together around this important issue
    - 2021….Focus Workshops being planned – dates to be confirmed by the e.o. 2020.
    - We hope "in collaboration with our partners in other regions"
  - Contribute to a simple solution, science driven and fit for the future

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