European Bioanalysis Forum

Bioanalysis of large molecules in a regulated bioanalytical environment – which is industry's challenge today?

Presented by Philip Timmerman, on behalf of EBF

at the EBF Focus Workshop

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

21-22 June 2017, Lisbon, Portugal

Disclaimer: not all ideas expressed in this slide deck have been discussed in the EBF. By presenting them at this meeting, they form a starting point for reflection and discussion in the scientific community.

The questions that bring us to Lisbon?

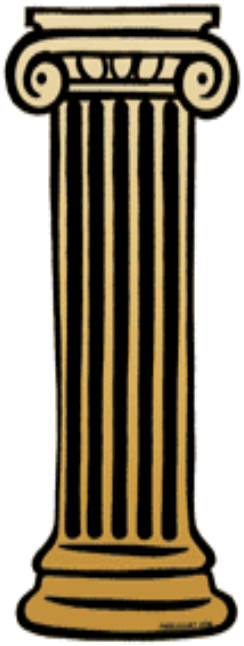
- How to define bioanalytical strategy around peptide and protein analysis in different stages of development
  - LBA?
  - CHROM?
  - When and why to use which technology?
  - What do reported concentrations mean (PK/PD, TK, PK,….)
- Update on the progress of peptide and protein analysis with MS based technology
- How to manage the science of peptide and protein assays using LC-MS?
- How to manage regulations of peptide and protein assays using LC-MS?

Semantics

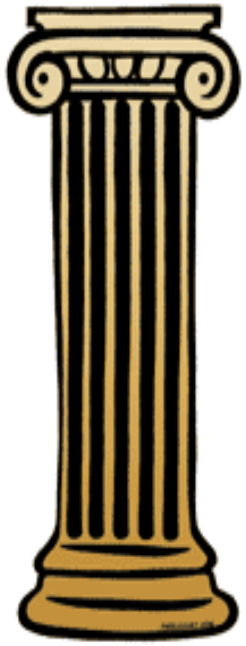
"Peptide and protein assays using LC-MS?"

….We seem to landing on "**Hybrid assays**"….

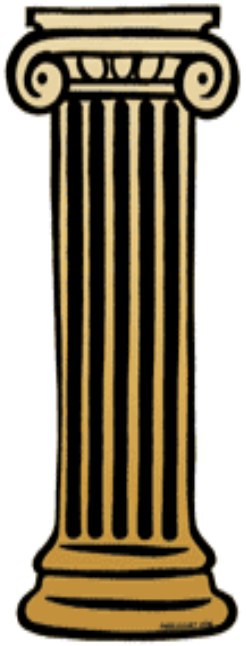
And inadvertently defining a 3rd pillar in the BA toolbox?



LBA



HYBRID



CHROM

Exactly 6 years back

European Bioanalysis Forum

Regulatory Challenges and Acceptance Criteria

First EBF reflections on Method validation criteria for peptide/protein analysis with LC-MS based techniques

Presented by Philip Timmerman, on behalf of EBF

at the EBF Focus Meeting – Large meets Small

21-22 June 2011, Brussels, Belgium

Drivers of the meeting in 2011

Changing environment

- Portfolios changing from NCE into NBE
- High pace of technological developments

Scientific challenges

- using multiple platforms yielding different concentrations for 1 ‘analyte’
- and how to interpret the data i.e. Biological readout vs analytical readout

Regulatory challenges

- Emerging Guideline in many regions
- But not following pace of changing environment

The Focus Meeting emphasised

1. Understand the science of your assay
2. Reflect on strategic use of LBA vs. MS-based technology
3. Recommend on regulatory requirements

Next 8 slides were copied from the 2011 Large Meets Small Focus Meeting, and remain actual in our discussions today: *Philip Timmerman (Janssen R&D for EBF) - First EBF reflections on Method validation criteria for peptide/protein analysis with LC-MS based techniques*

Website: http://bru2011.europeanbioanalysisforum.eu/slides

Points of attention - Regulations

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

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?

Points of attention - Regulations

Method Validation: experiments?

- Do we need to revisit relevance of the current validation experiments in the context of LmS?
  - Do we miss experiments/validation parameters?
  - Again, what about mixed technology methods (e.g. LBA prep + MS/MS detection?)
  - Or worse…..methods involving (enzymatic) digestion or tagging
- How to approach stability assessment and interpret them?

Study execution: acceptance criteria

- Similar discussion as for Method Validation
- Analytical design? Single or duplicate analysis? Etc…

So, still a lot of questions

The Small folks

- A lot of analytical chemistry-genes
- Underdeveloped biology-genes
- Organic chemistry is a hobby
- Get all excited by high-end analytical instruments
- Thinks (s)he understands LBA …… but may underestimate the challenge
- Eager to start analyzing peptides/proteins with MS/MS because because because…
- Stubborn and sensitive, as all bioanalyst

The Large folks

- A lot of biology and biochemical-enes
- Underdeveloped analytical chem try-genes
- Organic chemistry is a nightmar
- Convinced that automated pipet s are high-end analytical instruments
- Thinks (s)he understands chrom. Assays …… but may underestimate the challenges
- Not eager to dive into chromatography because because because….
- Stubborn and sensitive, as all bioanalyst

These challenges can only be overcome if we pool our experience and walk this trail together

Back to the Future

And we walked together…

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

…but also invented “hybrid assays”

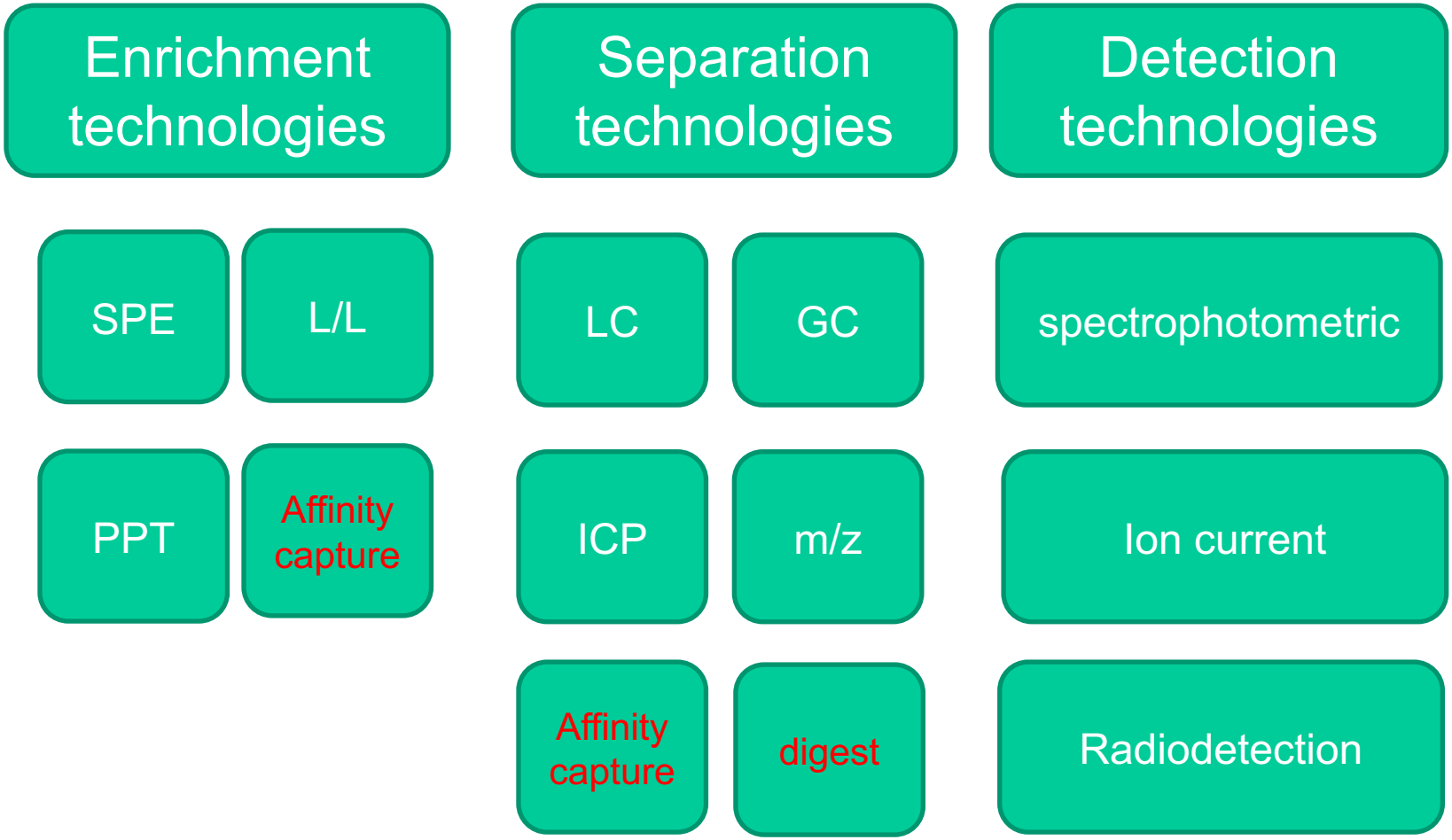
Hybrid techniques (from wikipedia)

……..

Combinations of the above techniques produce "hybrid" or "hyphenated" techniques. Several examples are in popular use today and new hybrid techniques are under development. For example, gas chromatography-mass spectrometry, LC-MS, GC-IR, LC-NMR, LC-IR, CE-MS, ICP-MS, and so on.

- Hyphenated separation techniques refers to a combination of two or more techniques to separate chemicals from solutions and detect them. Most often the other technique is some form of chromatography. Hyphenated techniques are widely used in chemistry and biochemistry. A slash is sometimes used instead of hyphen, especially if the name of one of the methods contains a hyphen itself.
- Examples of hyphenated techniques:
  - Gas chromatography-mass spectrometry (GC-MS)
  - Liquid chromatography–mass spectrometry (LC-MS)
  - Liquid chromatography-infrared spectroscopy (LC-IR)
  - High-performance liquid chromatography/electrospray ionization-mass spectrometry (HPLC/ESI-MS)
  - Etc….

But what makes a “hybrid assay” a “hybrid assay”?



Why would we call current peptides and protein assays ‘hybrid assays”?

- Because we use protein digestion?
- Because we use affinity capture as enrichment technologies?

And is there a real difference with legacy “hyphenated assays”?

- Why the difference with ion exchange sample enrichment?
- Why the difference with de-conjugation of conjugates?
- Why the difference with SPE?
- Etc…

Or, are we looking for an alibi to decide on acceptance criteria?

….and 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.

Interesting starting point for experts statisticians:

In summary

An invitation to reflect together on following 3 themes

- Hybrid assays: Do we need this 3rd category alongside LBA and CHROM assays?
- Harmonized Acceptance criteria for PK assays: Do we continue to need different LBA and CHROM (let alone add a 3rd cateory) acceptance criteria for define criteria for PK assays?
- 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?


EVERYTHING IS
POSSIBLE. THE
IMPOSSIBLE JUST
TAKES LONGER.