The sense and nonsense of quantitative analysis of biologics

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Why should we care?

EBF

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Introduction

A reflective essay on the role of (quantitative) PK analysis in the development of biologic drugs

- New drugs are evaluated based on their PK profiles
- Analytical trend: from Ligand Binding Assays toward LC-MS technology
- What is the driving force?
- Is this based on biochemical attributes of the analytes or does it emulate small molecule technology?
- Should we consider alternatives, more suited for biologic drugs?
- What do regulatory authorities require?
- Can we formulate recommendations for essential analytical platforms?

Why PK analysis?

- Determination of the fate of a substance (drug) administered externally to a living organism (LADME):
  - Liberation - release of a drug from the formulation
  - Absorption - process of the drug entering the circulation
  - Distribution - dispersion throughout the fluids and tissues of the body
  - Metabolism - transformation of a drug into metabolites
  - Excretion - elimination (or accumulation) of the drug from the body
- The rate at which a drug action begins and the duration of the effect (formulation of optimum dosing) - Study of *efficacy*
- Population PK - personalized medicine - personalized *efficacy*

→ PK analysis helps to understand and predict clinical efficacy

What is current practice?

(Pre-)Clinical development of biologics:

- PK analysis (what are we analyzing?)
- Emphasis on target binding and efficacy
- Immunogenicity (ADA) testing - effects on safety and efficacy

Physicians practice:

- "*If it doesn't work we give a little more; if it has adverse effects we stop medication*"
- Focus on the actual therapeutic dose range - 'safety and efficacy window'

Regulatory requirements?

Do regulatory authorities actually require PK testing?

- → Traditional small molecule drugs: Guidelines *how* to validate PK assays *when* you perform them...
- → ICH Topic S6 - CPMP/ICH/302/95: "*It is difficult to establish uniform guidelines for PK studies for biologics*"

  "*PK in SAD/MAD can be useful ... however (in tox studies) routine mass balance is not useful*"
- → WHO 2009 - Guidelines on evaluation of similar biotherapeutic products: "*The PK profile should always be investigated ... Special emphasis should be given to selection of analytical method, as assay methodologies suffer from limiting usefulness*"

- → What are the strengths and limitations of current analytical methods?
- → Do regulatory authorities require the use of specific analytical methods?

Analytical methods for PK

Common methods for PK analysis of biologics

- Immunoassays (ELISA, RIA, IRMA, MSD-ECL, Luminex, Gyros)
  - Proteins, Peptides, Conjugated proteins, Antibody drugs
- Hybridization assays
  - Oligonucleotide Therapeutics (asRNA, siRNA)
- Chromatography assays (HPLC, LC-MS/MS)

→ Trend to shift from Immunoassays to LC-MS/MS

→ Is this shift based on biochemical attributes of the analytes or is it an attempt to emulate small molecule technology?

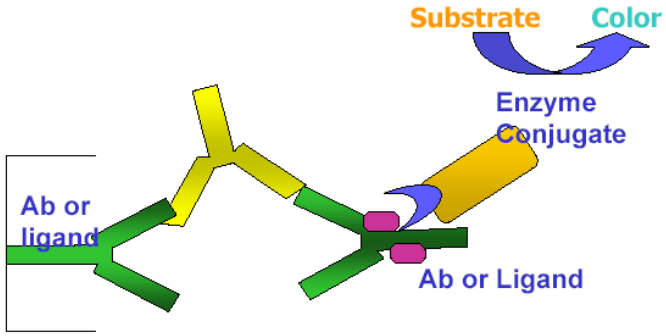
Driving force for analytical shift?

Strengths and limitations

Ligand Binding Assays

+ Sensitivity
+ Specific for structural conformation - correlation with efficacy
+ Combination of epitope recognition
+ Idiotype specific (Antibody drugs)
+ Avidity - synergy of the affinity sites
+ Specific assays for total, target-bound or free analyte

- Relative variability/imprecision - no internal standard
- Dependent on availability (and changes) of critical reagents
- Sensitive to structural changes
- Influence of anti-drug antibodies
- Time consuming assay optimization

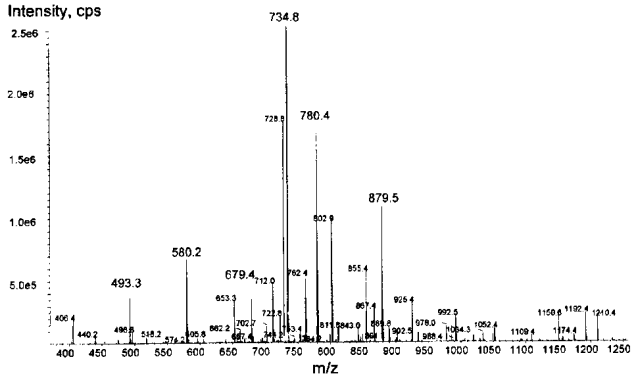


Strengths and limitations

Chromatography assays - LC-MS/MS

+ Quantitative assays
+ Good precision - use of internal standards
+ Relatively fast assay optimization

- Sensitivity
- Focused on primary structure and signature peptides - digestion
- Limited association between concentration and activity
- Sensitive to biotransformation of the parent drug
- Analysis of free drug only
- Production of internal standard

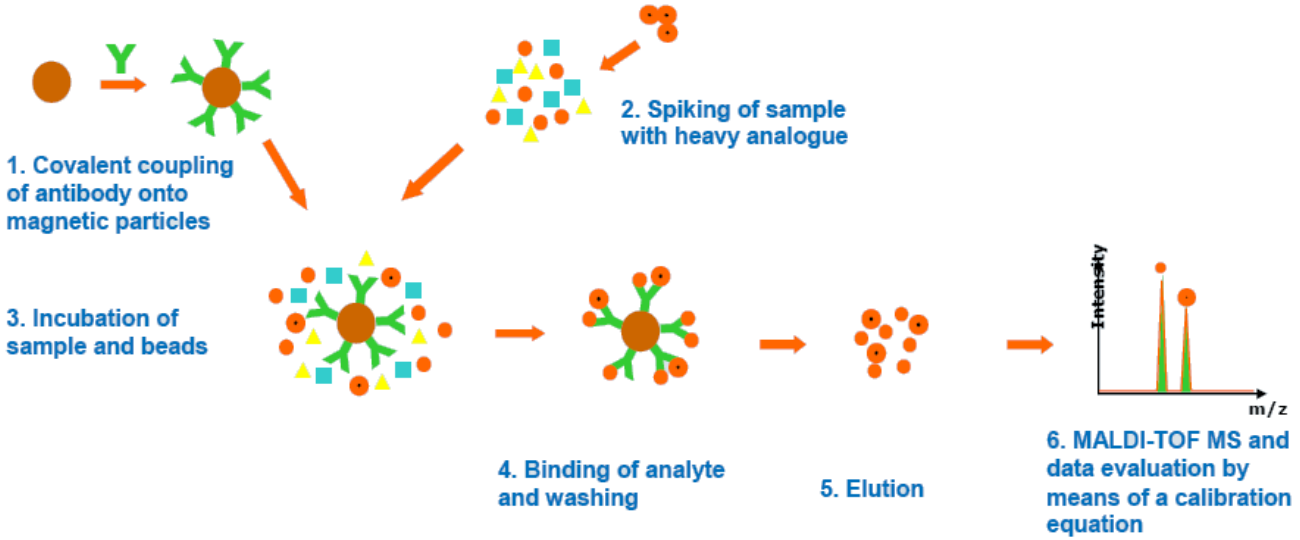


Best of both worlds?

Quantitative Immuno-MALDI-TOF MS

(K. Sparbier, et al.; Association of Biomolecular Research Facilities 2008, Salt Lake City, Utah)

+	Quantitative assay	-	Sensitivity
+	Internal standard (precision)	-	Specific antibody reagents required
+	Detection of structural epitopes		



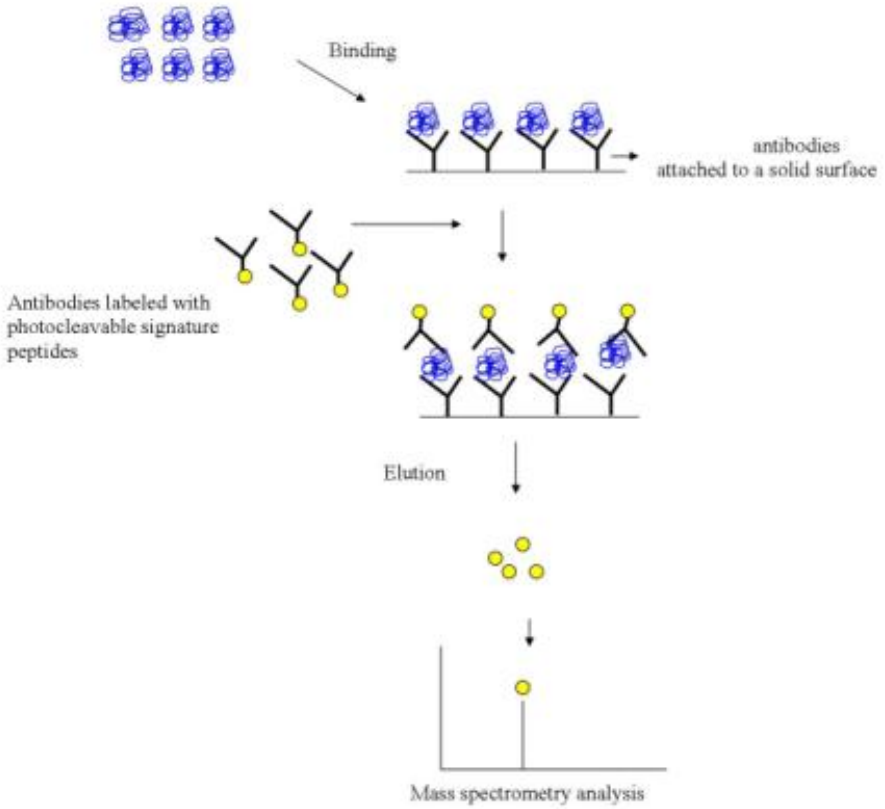
Best of both worlds?

Universal Immuno-MS method

(Proteora; SciClips Open Innovation Vol. 1, 2009, page 2B)

+ Quantitative assay
+ No digestion or purification (precision)
+ Universal reagent
+ Detection of structural epitopes

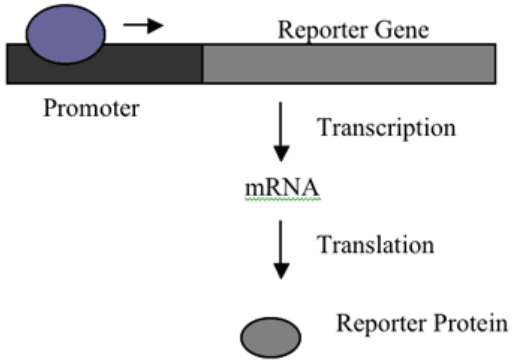
- No internal standard
- Specific antibody reagents required



Alternative methods

- Binding to target (circulating antigen, cells, receptors)
  - Immunoassays for total, bound and free analyte
  - Flow Cytometry (receptor binding assays)

- Potency assays → investigating actual efficacy of the drug
  - Classical cell based assays
  - Reporter gene expression
  - → Establish PK profile based on elicited effect



Regulatory requirements

→ Regulatory authorities actually *require* potency/efficacy assays for research of biosimilar drugs

- Examples of guidelines:
  - ICH Topic S6 - CPMP/ICH/302/95 - Focus on safety, biological activity and immunogenicity
  - EMEA/CPMP/3097/02 - Guideline on comparability of medicinal products containing biotechnology-derived proteins as active substances
  - FDA 21 CFR 610.10 - Regulatory requirement for determining potency - Unique to biologics!

→ Focus on purity, safety and potency

Bioactivity assays

Bioactivity/Potency methods

- Can be used for 'total' PK profiling
- Detect active biotransformations - alternations, modifications, metabolites with impact on bioactivity
- Useful for biocomparability studies
- Functional for neutralizing antibody detection

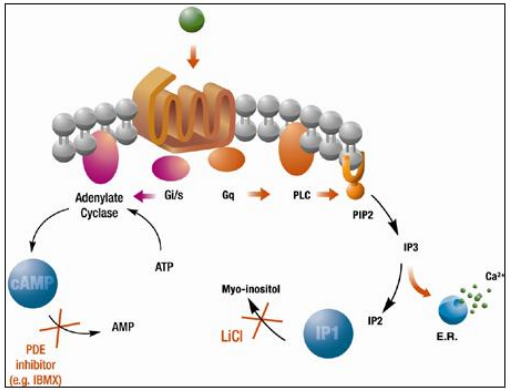
\+ Relevance to drug bioactivity

\+ Cell-based: binding + signal transduction

\+ Normalized to internal standards and International Reference standards

\- Less sensitive than LBAs

\- Relatively more variable



Bioactivity of isomers

Antiviral potency of monopegylated Interferon alpha-2a (PEGASYS)

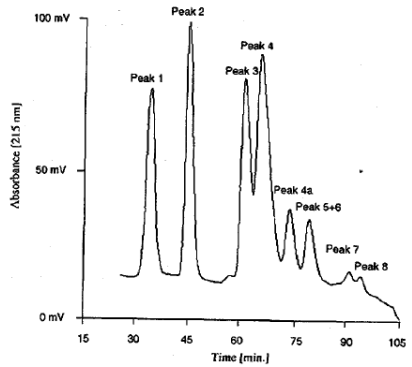
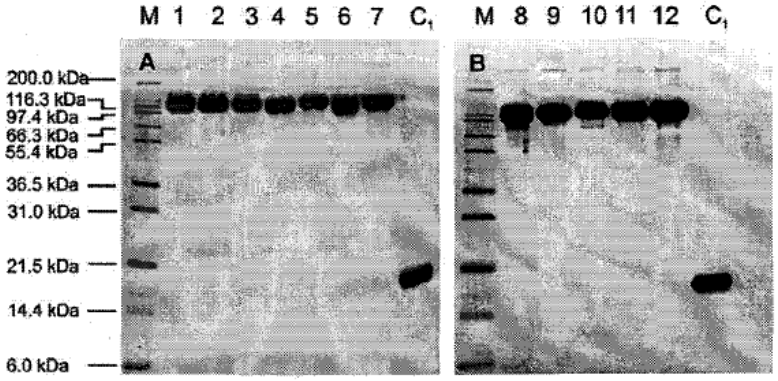


Fig. 1. Analytical IEC-HPLC of 180 µg PEG-IFN.



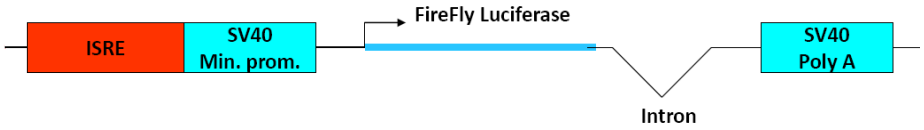
Identified PEG-peptides and the antiviral activities of individual positional isomers

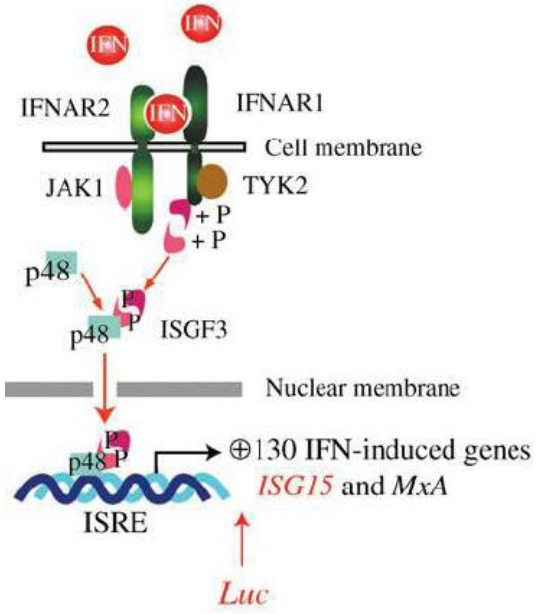
	Identified PEG sites in peptide map			Antiviral activity
	PEG site	Missing peaks	Sequence	U/µg
PEG-IFN				1061 ± 50
Peak 1	K31	A, E	24–49	1818 ± 127
Peak 2	K134	I, I′	134–164	1358 ± 46
Peak 3	K131	C	122–131[a]	761 ± 97
Peak 4	K121	B, C	113–131	339 ± 33
Peak 4a	K164	[b]	134–164[a,b]	966 ± 107
Peak 5	K70	D, F	50–83	600 ± 27
Peak 6	K83	D, H	71–112	463 ± 25
Peak 7	K49	E, F	32–70	513 ± 20
Peak 8	K112	B, H	84–121	468 ± 23

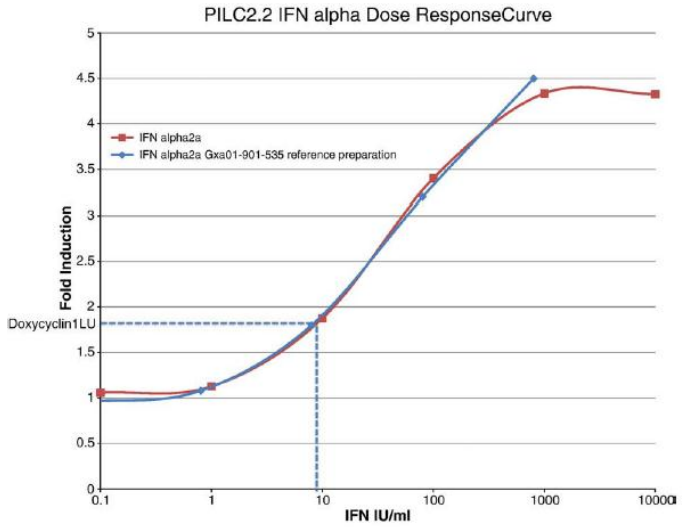
S. Foser et al. / Protein Expression and Purification 30 (2003) 78–87

Reporter gene technology

Reporter gene Potency assay for Interferon alpha: PK and NAb assays







Ch. Lallemand et al., J Immunol Meth 356 (2010), 18-28

K. Bendtzen, J Interferon & Cytokine Research, Vol 30 (2010), 759-766

Recommendations

Recommendations for PK assays for biologics:

- Bioactivity/Potency assays - activity-based PK profile
- Receptor binding assays - target bound drug
- Immuno-MS - structural epitopes + internal standard
- Immunoassays (LBA) - structural epitope recognition
- LC-MS/MS assays - precision through internal standard


Thank you!
You have so much potency!