Intertek

Valued Quality. Delivered.

Alternative Methods to LC-MSMS and Immunochemistry in Bioanalysis

European Bioanalysis Forum 2014

Ashleigh Wake

Intertek Pharmaceutical Services

Introduction

LC-MSMS and ELISA based techniques...

When they go well they leave us feeling like this…

But sometimes we end up feeling like this!

The Answer?

Use of non-traditional bioanalytical techniques in some situations might provide the answer.

- Quantitative NMR
- ICP-MS
- CGE

CASE 1: Quantitative Bioanalytical NMR

Intertek
Valued Quality. Delivered.

Reasons for PEGylation well documented:

Present many bioanalytical challenges

LC-MSMS and Ligand Bind Approached typically applied but have problems…

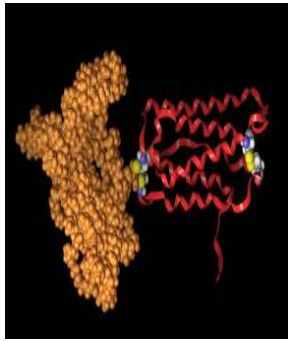
LC-MSMS	LBA
Intact mass polydispersity Charge state distribution Isotope distribution Reduce “sensitivity”	Antibody Specificity
Can require complex sample preparation techniques.	PEG.. Low immunogenicity so difficult to produce significant levels
Can require the need for non PEGylated specific peptides.	

CASE 1: Quantitative Bioanalytical NMR

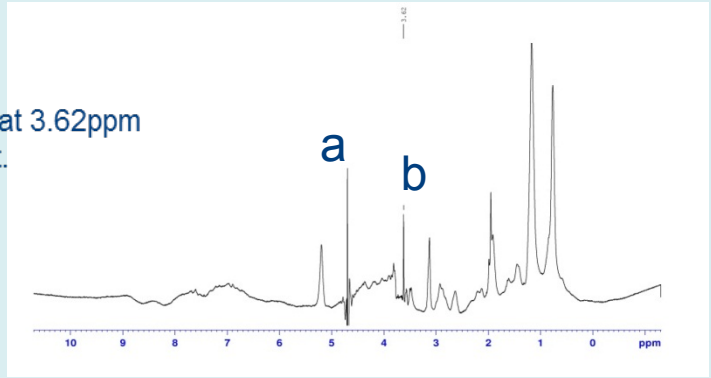
Intertek
Valued Quality. Delivered.

Case Study

- A PEGylated protein with a PEG molecular weight of ~400g/mol was examined in plasma using quantitative NMR on a Bruker Advance 500MHz Spectrometer against a suitable internal standard.
- Typically the LOD and LOQ is dependent on the molecular weight of the PEG species .
- Not specific likely to include contribution from any metabolites (if relevant) and free PEG.
- In general sensitivity would be increased by moving to a 600MHz. Or simply scanning longer.



(a) the suppressed water peak (b) the PEG peak at 3.62ppm which is due to the PEG protons in the repeat unit.



CASE 1: Quantitative Bioanalytical NMR

Intertek
Valued Quality. Delivered.

- High resolution NMR can offer direct quantitative bioanalytical approaches with minimal sample preparation, facilitating low levels of PEG species to be observed.

Results:

- An LOQ of 0.09mg/mL was determined for this product in plasma samples
- Accuracy at LOQ >95%
- Precision %CV at LOQ < 3%
- **NO SAMPLE EXTRACTION, ADD INTERNAL STANDARD AND GO!**

CASE 2: Bioanalytical ICP-MS

Intertek
Valued Quality. Delivered.

Inorganic Pharmaceuticals:

- Vanadium – Insulin like properties
- Manganese – salen and salphen - oncology
- Platinum – Cisplatin etc.…

Case Study: Manganese in the form of metalloporphyrins

CASE 2: Bioanalytical ICP-MS- Determination of trace metals in plasma.

Intertek
Valued Quality. Delivered.

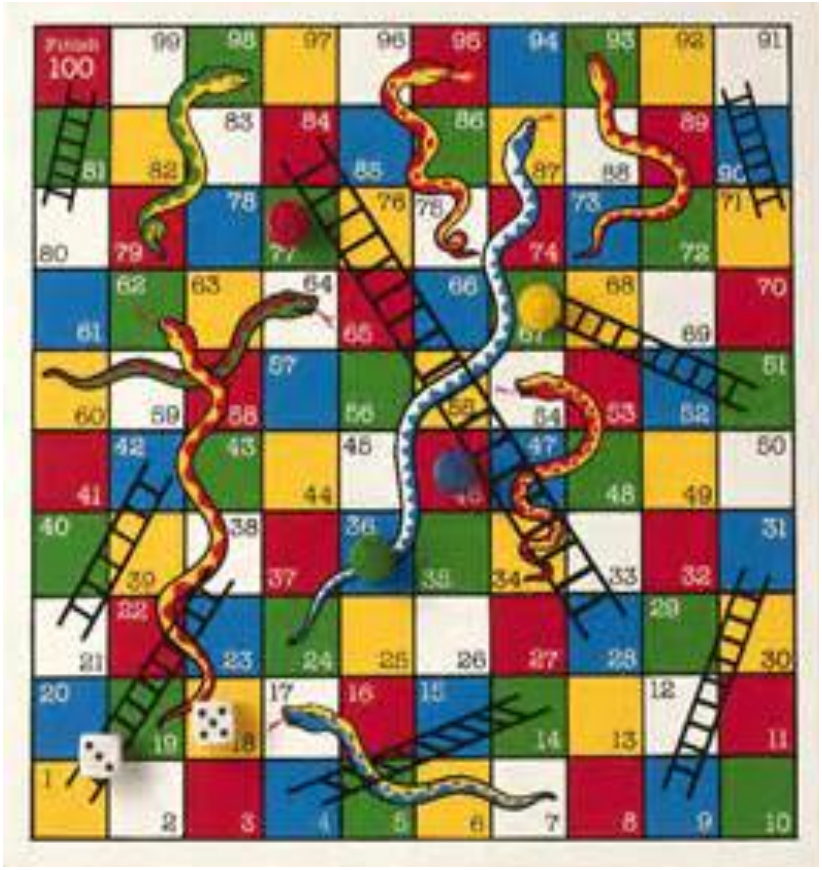
Benefits:

- Exceptionally low detection limits for most elements with few interferences even with very simple preparation.
- Simply an Internal (element specific) internal standard added to an aliquot of the sample plasma i.e: for Manganese Internal Standard = Germanium, for Platinum Internal Standard = Iridium
- The sample solutions are sonicated to reduce the particle size of any insoluble material that may be present. prior to analysis by ICP-MS.
- Mean Mn levels per sample were determined across the multiple samples at levels between 15 and 20 ng/mL. (approx. <1ppb in solution)
- Can be applied to tissues, body fluids, or practically any other sample matrix.

Positive	Negative
Exceptional Sensitivity	Destructive
Can be used on many matrices	Limited sample application
Near Universal Approach.	

Newest not always the best

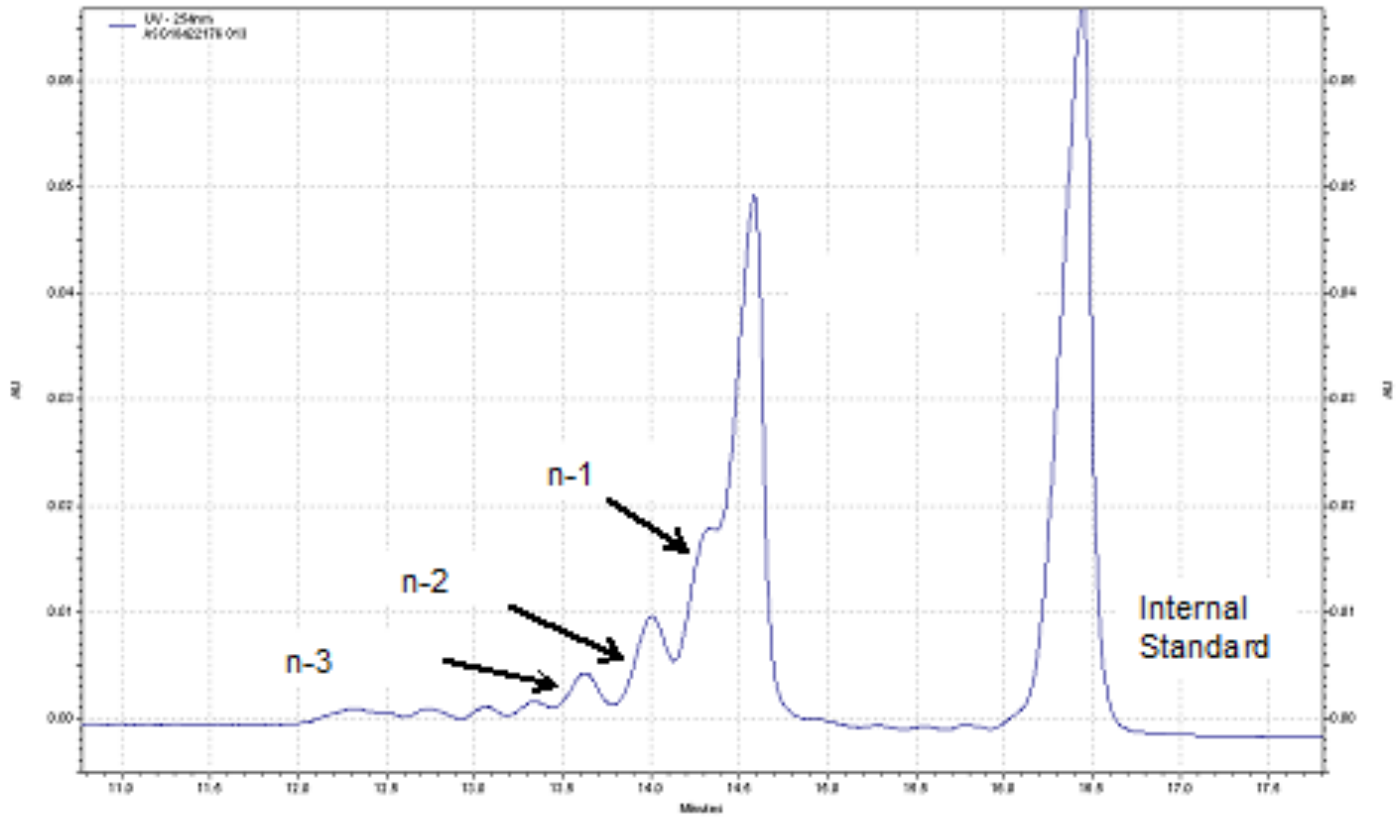
Intertek
Valued Quality. Delivered.



CASE 3: Oligonucleotide Bioanalysis

Intertek
Valued Quality. Delivered.

- Oligonucleotides are very prone to metabolism through truncation of the parent, giving n-1, 2 ,3 etc.
- For quantitation of intact species LC-MSMS is extremely powerful, however not easy to resolve “metabolites.
- CGE however can provide good separation of species.
- Intertek have successfully developed and validated oligonucleotide CGE methods to support clinical/ preclinical studies reaching limits of quantitation in the ppb range where the parent is resolved from n-1 and further metabolites.
- So far these have been based purely on UV detection, with the advancements in online CE-MS instrumentation the next challenge will be to see how low LOQs can be pushed.


n-1
n-2
n-3
Internal
Standard

Intertek

Valued Quality. Delivered.

Thank you and any questions:

ashleigh.wake@intertek.com

Biopharmaceutical Services Leader