Solution Reference Standards for Protein Based Pharmaceuticals

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Introduction

The increasing prevalence of monoclonal antibodies and other biopharmaceuticals as drugs and drug candidates presents new challenges in pharmaceutical analysis. Many of these molecules are hygroscopic, air sensitive, highly potent, and can be impossible to isolate as neat powders without loss of biological activity. The traditional approach of using neat powders as the reference standards is therefore not the best practice for many biomolecules. Alternatives, such as lyophilized materials, are generally stable but present issues such as high cost and variability in material content from vial to vial. Lyophilized vial manufacturing can also have a higher rate of failure, which is problematic for high-value source materials such as biologics.

A solution-based reference standard is an alternative format that mitigates the handling issues associated with these materials and promotes consistency in testing. Storage in an ampouled format maintains concentration, protects from air and light, and can promote stability.

"Special" Qualities of Protein Based Pharmaceuticals

- Hygroscopic
- Air sensitive
- Thermally labile
- Electrostatic and/or flocculant
- Potent
- Unstable or cannot be isolated in powder form
- Packaging format typically must be compatible with biological assays

Solution Based Protein Standards - Ampouled Format

- Eliminates handling of difficult powders during routine use
- Can be prepared under inert conditions – glove box
- The protein need not be isolated as a powder
- Ampouled format with argon headspace provides inert long term storage environment
- Amber ampoules protect from light
- Cryule ampoules can be stored in the sub-freezer with little to no breakage
- Labor savings due to reduced material handling in the analytical lab
- Handling of dilute solutions is safer than powder for potent compounds
- Concentration in ampoule is more consistent than reconstituted lyophilized powders. Solution can be quantitatively transferred
- Potential cost savings from fewer out of specification or out of trend results due to reduced variability in reference standard compared to lyophilized powders
- Greater consistency achieved by using the same solution standard over a longer period of time - efficiency gains and fewer failed batches due to standard preparation errors = $ saved
- The cost of preparing an ampouled solution standard is significantly lower than lyophilization

Example1: Monoclonal Antibodies

- Growing number in the development pipeline - a focus of pharma R&D worldwide
- Development of reference standards for MAB API's present unique challenges compared to small molecule
- Critical to develop the reference standard during early phases of product development
- Accuracy and stability of standards are vital
- Cryule ampoules can be stored deep frozen – a requirement for long term stability of monoclonal antibodies

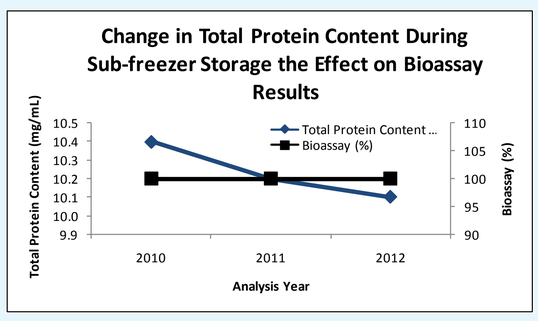
Monoclonal Antibody 1

- Cerilliant prepared and filled a MAB solution for Lilly
- Non-sterile fill
- 0.25 mL per container
- 2 mL cryule ampoule
- Argon headspace
- Sub-freezer (-70°C to -80°C) storage

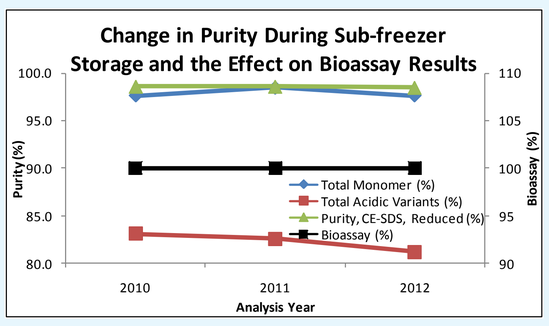
The Primary use for this material is as a Reference Standard in bioassay evaluations for API's and drug products

Real Time Stability Results

	Date	Total Protein Content (mg/mL)	Total Monomer (%)	Purity, CE-SDS, Reduced (%)	Total Acidic Variants (%)	Bioassay (%)
Initial Evaluation	2010	10.4	97.6	98.6	83.1	100
First Re-evaluation	2011	10.2	98.5	98.6	82.6	100
Second Re-evaluation	2012	10.1	97.6	98.5	81.2	100



The minor change in the Total Protein Content had no effect on the Bioassay results



The minor changes in the Purities had no effect on the Bioassay results

Benefits of Monoclonal Antibody Solution Standards

- Stability in the ampouled solution format has been demonstrated
  - The example ampouled solution standard was stable for 2 years
  - Biological activity is retained because the monoclonal antibody is not isolated as a neat material
  - Solution format minimizes manipulation of the monoclonal antibody and the number of freeze thaw cycles
  - The ampouled solution reference standard can be prepared under non-sterile conditions
- Cryule ampoules offer a secure option for long term sub-freezer storage of aqueous solutions

Monoclonal Antibody Solution Standards: Conclusions

- Solution-based protein standards can be prepared, packaged and stored in a manner that preserves their integrity
- Monoclonal antibody solution standards provide reliable and reproducible results over multiple years
- The ability to preserve monoclonal antibody reference standards (primary standard) is critical to allow a consistent baseline of measurement from development and into commercial manufacturing
- Use of a consistent, reliable and stable solution standard provides significant cost benefits for these high dollar and high potency classes of materials

Ampouled solutions present a viable "solution" for the challenges associated with reference standards for protein based materials

Answers That Matter.

Cerilliant® Analytical Reference Standards
a SIGMA-ALDRICH® company

Example 2: Biosynthetic Human Insulin (BHI) Solution Check Standard

Biosynthetic Human Insulin (BHI): Prior to the Solution Check Standard

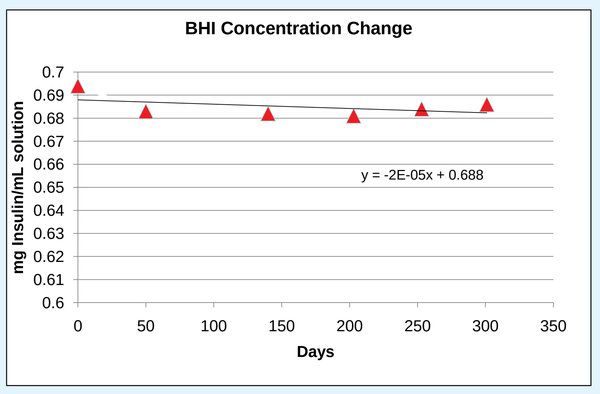
- Every 90 days, testing labs would open 90 vials of lyophilized Corporate Reference Standard
- The vials were reconstituted, pooled, subdivided into 90 glass vials, stoppered, crimped, and frozen
- The solution was tested to ensure proper concentration was achieved
- The vials were frozen and used over the next 90 days to prepare check standards for HPLC analysis

Solution Check Standard Preparation

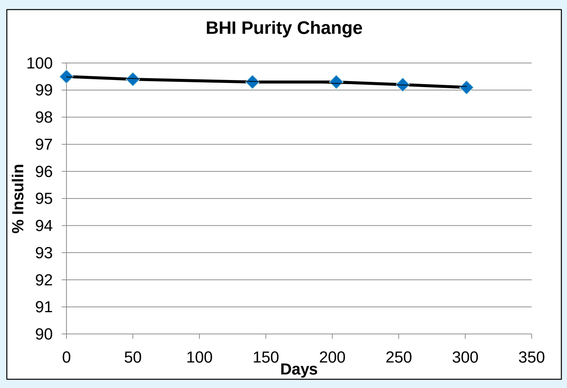
- A solution standard was developed and qualified by Cerilliant to match the concentration and matrix of the pooled check standard solution
- The standard was prepared gravimetrically – with weigh tapes providing traceability of preparation
- Material was handled in a glove box
- Dispensed immediately into amber ampoules and sealed under argon
- Packaged in glass flamed-sealed ampoules compatible with freezer storage.
- Stored in freezer
- Fill volume sufficient for single day use
- Cerilliant demonstrated qualification to perform compendial methods to release and monitor stability
- Standard was put on stability for 300 days – tested for concentration and purity by compendial methods
- Lilly labs only need to open the ampoule and transfer to autosampler vials – Time <5 minutes
- Snap-N-Shoot®!

Real Time Stability Results

Day	% Insulin	Concentration (mg/mL)
0	99.5	0.694
50	99.4	0.683
140	99.3	0.682
203	99.3	0.681
253	99.2	0.684
301	99.1	0.686



Based on the best-fit line, the concentration of the BHI Check Standard solution decreased 0.87% over 300 days



Based on the best-fit line, the purity of the BHI Check Standard solution decreased 0.36% over 300 days

Changes are within acceptable range for the intended use of the check standard

Benefits of the BHI Solution Check Standard

Before	After	Outcome/Benefit
~400 vials of Corporate Reference Standard consumed per year by each lab	A single batch of ampoules can supply all testing labs for a full year	Estimated cost savings of the CRS is more than $50,000 per year
Time consumed by the lab to prepare and test the pooled solution every month	Labs only need to open ampoules and transfer to autosampler vials	Many hours of lab resources saved every 90 days
Control charts of the check standard results over time could shift every 90 days	Check standard batches last almost one year	Able to monitor over 300 days instead of 90 days
Corporate Reference Standard batch stock out was accelerated by non-required use	Corporate Reference Standard batches last longer	Less frequent batch replacement reduces impact on testing labs

Biosynthetic Human Insulin (BHI): Conclusions

- Solution-based protein check standards can be prepared, packaged and stored in a manner that preserves their integrity
- The Biosynthetic Human Insulin solution check standard provides a reliable and independent way to track method performance over a longer period of time compared to the lab-prepared standard
- This is critical so that high-volume production of a product critical to patients can be done in a consistent manner
- Use of a customized check solution standard provides large cost and time savings benefits for this high-volume application