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Review Article

Identifying Trending Issues in Assay of Peptide Therapeutics During Stability Study

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Abstract

Peptide therapeutics represent a promising class of drugs with diverse applications, but their analysis requires addressing the unique challenges associated with these biomolecules. Establishing appropriate quality control metrics and benchmarks, as well as ensuring reproducibility between day, analyst, instrument and different laboratories is essential for reliable peptide analysis. In this article we discussed the analytical challenges related to hygroscopicity, weighing of peptide working standard, stability, and standardization is crucial for obtaining accurate and reproducible results in the analysis of peptide therapeutics, which is essential for their development and quality control. The use of well-characterized, lyophilized peptide working standards can improve the accuracy and precision of peptide quantification.

Keywords: Weighing Peptides, Static charge in peptides, Lyophilized working standard, Assay troubleshoot, OOT

Introduction

Peptide therapeutics are peptides or polypeptides that are used for the treatment of diseases. They can mimic the functions of naturally occurring peptides like hormones, growth factors, neurotransmitters, and anti-infectives. The peptide therapeutic market is growing, with over 100 FDA-approved peptide drugs and more than 200 peptides in clinical development. The biggest concern in the peptide analysis is getting reproducibility in the results. Identifying and handling out-of-trend (OOT) results is a critical issue in the stability study and shelf-life prediction of peptide therapeutics. OOT results are stability data points that deviate significantly from the expected degradation trend, often due to analytical errors, sample mishandling, or other sources of variability. Failure to identify and remove OOT results can lead to inaccurate stability modeling and incorrect shelf-life estimates.

In this article we will study the probable root cause of assay variation by RP-HPLC, UPLC (Chromatographic techniques) in peptide therapeutics. The inherent heterogeneity of peptides, complex sample preparation, and analytical factors can all contribute to day-to-day variability in peptide assays. Figure 1 represents the Interday variability cause and working standard comparison to overcome the Interday variability.

Peptides Characteristics

Peptides containing certain amino acids like Cysteine, Methionine, or Tryptophan are more susceptible to oxidation, which could be the leading cause of variation in results [1]. Hydrophilic peptides that elute too early from chromatographic columns can have higher variability [2].

Sample Preparation

Improper dissolution and handling of hydrophobic peptides can lead to precipitation and assay variability [2]. Different sample preparation methods like enrichment strategies (e.g., nanoparticle, glycopeptide, immunoaffinity) can impact the reproducibility [2,3]. Sample preparation for majority of therapeutic peptides is simple and involve the single dilution to achieve the test concentration with appropriate diluent. Some peptides analyzed as is because the label claim of API in sample concentration is low. Still the assay variation on day-to-day basis can be observed that could only result from standard preparation [1].

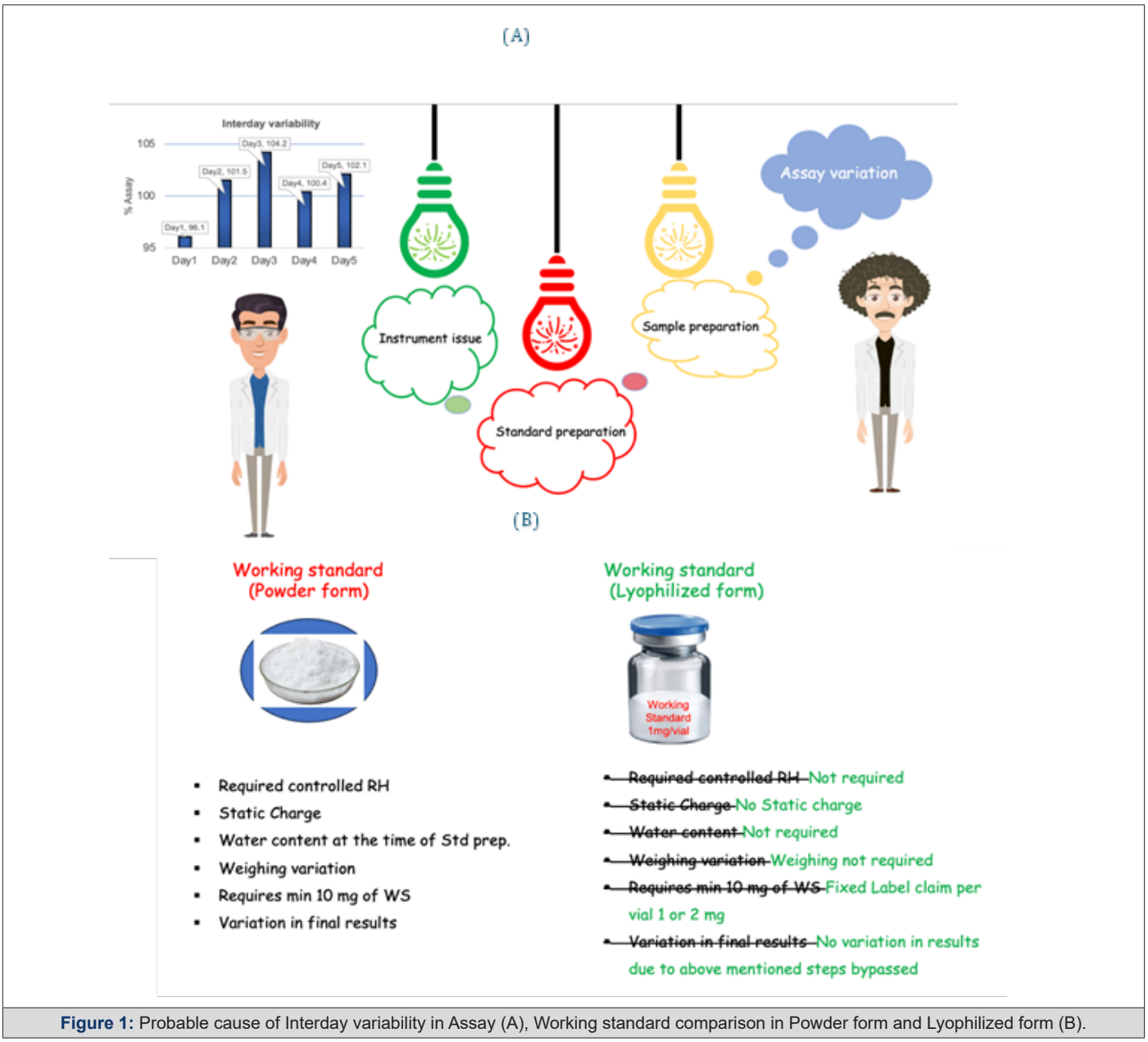

Figure 1: Probable cause of Interday variability in Assay (A), Working standard comparison in Powder form and Lyophilized form (B).

Standard Preparation

The another most important factor that is contributing to the variation in assay is the standard preparation. Peptides are often highly hygroscopic, meaning they have a tendency to absorb and retain moisture from the environment [4]. The high hygroscopicity of peptides can lead to physicochemical changes that affect their stability during storage, accelerating chemical and microbial degradation [5]. Hence the peptide standards should be packed in appropriate container and stored at the prescribed storage condition [6]. The container closure system used for peptides should be suitable to protect against moisture uptake, such as including appropriate desiccants or using storage under inert atmosphere. For storage, standards are often recommended to be kept under refrigeration (5°C ± 3°C) or freezing conditions (-15°C or below) to minimize hygroscopic effects and improve long-term stability. Thawing in a desiccator should be carried out for at least one to two hours following removal from the refrigerator. In the balance room, the humidity level should be kept between 25-35 % while weighing [6].

Weighing peptides accurately can be challenging and time consuming due to their tendency to accumulate static charge, which causes the peptide powder to stick to surfaces and become difficult to handle.

Proper control of static charge is crucial for accurate peptide weighing, especially during preparation of standards or formulations where precise quantities are essential [7-9].

Here are some key points regarding the weighing difficulties associated with static charge in peptides:

1) Peptides, being organic compounds with polar groups, can readily accumulate static charge through friction, making them electrostatically attracted to weighing vessels and spatulas [7-8].

2) This static attraction leads to peptide material sticking to the surfaces, resulting in incomplete transfer and inaccurate weighing [7-8].

3) The extent of static charge buildup depends on factors like peptide sequence, length, and environmental conditions (humidity, temperature) [7].

4) Highly basic peptides with a greater number of positively charged residues (Arg, Lys, His) tend to exhibit higher static charge and more weighing difficulties [7].

5) Low humidity environments exacerbate static charge problems, as the lack of moisture prevents charge dissipation [9].

To overcome or minimize static charge issues during peptide weighing:

1) Use anti-static weighing equipment like ionizing air guns or static discharge units to neutralize charges [9].

2) Perform weighing in a humidity-controlled environment or use anti-static agents [9-10].

3) Handle peptides with non-insulating tools like ceramic spatulas or metal micro spatulas with rubberized grip [9].

4) Eliminating weighing paper/boats by direct dosing into a volumetric flask reducing the potential for errors due to powder spillage during sample transfer and back weighing of weighing papers and calculations become obsolete.

Water Content

Peptides are often highly hygroscopic, meaning they have a tendency to absorb and retain moisture from the environment. The water content in peptides is a critical factor that needs to be carefully monitored and controlled to ensure the stability and accurate quantification of peptide-based products. The water content should always be ascertained during standard preparation in order to establish potency for the assay calculation [5]. Hence water content of reference standard/working standard should be performed in duplicate. Karl Fischer Titration is a widely used method for quantifying the water content in peptides. Coulometric Karl Fischer titration can also be used in combination with a heated oven to release the water from the peptide sample and found the most accurate and reproducible method for water content determination in peptides [4,11].

By applying the above-mentioned procedures if variation still persists than use of lyophilized standard vials may be used to nullify the standard preparation errors.

Lyophilized Peptide Working Standards

Lyophilized peptide working standards are essential for accurate analysis and quality control of peptide therapeutics.

To prepare the working standard lots for lyophilization, peptides were individually solubilized at concentrations between 1 and 5 mg/g in water for injection. The solubilized peptide solutions were dispensed in 1-g aliquots into type I amber glass vials and sealed with an appropriate stopper. After optimizing the lyophilization cycles final lyophilization of the working standard lot were performed. After completing lyophilization the working standard vials were stored at -20°C. Working standard qualified against the reference standard w.r.t identify (HPLC, LCMS, NMR, AAA), purity (TFA, Acetate, residual solvents, RS by HPLC) and strength (assay by RP-HPLC and water content by KF coulometer) [12-14]. After standardization the working standard the final potency of the working standard was determined per vial (peptide in mg/vial).

Benefits of the Lyophilized Working Standard

Improved Stability: Lyophilization (freeze-drying) enhances the stability of peptides by removing water and reducing chemical and microbial degradation. The dry, lyophilized powder form is more resistant to hydrolysis, oxidation, and other degradation pathways compared to liquid formulations.

Ease of Handling and Storage: Lyophilized peptides are easier to handle, and store compared to working standard in the powder form.

Accurate Quantification: It allows for the preparation of standardized solutions with known concentrations, which is crucial for analytical applications and quality control.

Reduced Contamination Risk: It eliminates the possibility of cross-contamination when transferring and weighing.

Less Time Consuming: In order to reach the ideal standard concentration, it entails immediate reconstitution through diluent, doing away with the need for water content analysis waiver and weighing time.

Cost Effective: Lyophilized working standard vials contain 1-2 mg of peptide, which can be used to make 1 µg/ml to 2000 µg/ml of standard solution. Conventional weighing requires more drug substance for standard preparation and water content determination, which raises the cost of the product.

Conclusion

Day to day variation in the assay of peptide therapeutics contributing from instrument, Sample preparation and Standard preparation. However, on evaluating all the data if no variation seen in sample area in interday analysis and system suitability also meets the acceptance criteria (bracketing standards) then only standard preparation comes into the question mark. Since peptide contains the static charge hence proper control of static charge is crucial for accurate peptide weighing, especially during preparation of standards. Anti-static weighing equipment (ionizing air guns or static discharge units), relative humidity and water content should be taken into consideration while standard weighing. By following the above-mentioned practices if variation still remains than lyophilized working standard which bypass the weighing steps, relative humidity and water content determination at the time of standard should be taken into the consideration for the analysis to overcome the day-to-day assay variability issues.

Conflict of Interest

None.

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