Tadalafil: A Case Study for the Development of Stable Solution Reference Standards for Pharmaceutical Applications

Lilly Authors: Matthew Borer, Michael Julius, David Lytle
Cerilliant Authors: Tamara Tarbox, Isil Dilek, Uma Sreenivasan, Mitzi Rettinger

Reprint from 11th International Reference Standard Symposium
September 2012

Introduction

Reference Standards are a critical part of the control strategy to ensure the quality, safety, and efficacy of pharmaceuticals. Routine QC of pharmaceuticals requires preparation of working solutions from powder reference material on a routine basis. These solutions are typically prepared in volumetric flasks and stored for short intervals in the laboratory. Weighing of materials for standards in the lab can be time consuming, especially when materials are hygroscopic, toxic, or controlled. One source of lot-to-lot discrepancies in release testing is variation in the reference standard working solution which can result from weighing and dilution differences between batches. A solution-based reference standard packaged in a format that preserves concentration and integrity of the material provides a convenient, consistent and cost-effective alternative to preparation of working reference standard solutions in the analytical lab.

Working solutions prepared from neat reference materials can present several challenges

- Solutions prepared by weighing neat materials and diluting in volumetric flasks
- Weighing small quantities accurately is time consuming and costly (estimated ~ 45 minutes per method execution)
- Accurate weighing of small quantities may require special balance enclosures and micro-balances
- Requires more reference material over time and more frequent batch replacement – cost impact
- Solutions stored in volumetric flasks may have limited shelf life due to evaporation and/or degradation. Shelf life for most working standards is typically days or weeks
- Hygroscopic, electrostatic, volatile, and difficult to handle materials pose handling and repeatability challenges.
- Special handling is also required for toxic, potent, or hazardous substances
- Other special equipment such as glove boxes and static control may be required
- Regulatory requirements may require detailed and time consuming paperwork to track material usage and disposition
- Correct preparation of the standard can be a factor in inter-lab variability
- Inconsistent preparation of working standards can lead to incorrect failure of a production lot, OOS investigations and batch rejection. Significant monetary impact

Alternative Approach: Qualified working standard solution in inert sealed ampoule format

- Larger batches – larger weighings – greater accuracy for the filling solution
- Ampoule format prevents changes in concentration due to evaporation
- Inert atmosphere promotes long term – years of stability
- Ampoule format prevents cross-contamination
- Standard preparation time reduced in the analytical lab
- If prepared at the working concentration, can use directly. If dilution required, preparation is reduced to quantitative transfer from ampoule
- Handling: The pre-diluted solution provides safety in handling of toxic, potent, or hazardous materials in the analytical lab
- Qualification to the primary standard provides traceability and consistency in results year after year
- Use of same lot over extended time significantly reduces variability of the reference – particularly for difficult to handle materials
- Fewer failed batches due to variability of the standard – critical for high dollar and/or difficult to handle materials
- Less paperwork associated with handling the pre-diluted solution – convenience and cost savings
- Exemptions can be obtained for US DEA & Health Canada regulated materials when in solution format – reduced paperwork eliminates regulatory burden for laboratory
- Proven track record in other industries – clinical/forensic toxicology, clinical diagnostic, and environmental

The Case for Tadalafil

- Material usage is tightly controlled and requires documentation of secure storage and traceability of usage.
- The material is electrostatic and requires special handling during the weighing of assay standards.
- An ampouled working solution standard for use in routine QC of tadalafil would provide cost, efficiency, and security benefits.

By presenting the reference standard as a low concentration solution in acetonitrile, there is a decreased incentive for the reference standard to be diverted for inappropriate uses. For example, reference standards are not suitable for human consumption. Materials with a propensity for this type of diversion require special handling requirements, such as chain of custody and accountability. These requirements are not required for the solution, resulting in:

- Reduced employee time completing and verifying paperwork required for special handling.
- Reduced shipping costs and time due to not needing special tamper-proof packaging for dispensing and shipping.
- Reduced analyst time at the bench for completing and verifying paperwork required for special handling. This is in addition to reduced analyst time to prepare the sample by weighing rather than a simple serial dilution.

Overall cost reduction is estimated as approximately $40,000 per year!

Solution Design

Method considerations: The HPLC/UV method for tadalafil requires two standards to be prepared by weighing a powder. The working solution requires dissolution in acetonitrile (ACN) followed by dilution with aqueous diluent. The volumetric solution has been shown to be stable for two days. The powder is electrostatic and difficult to handle with greater potential for day to day variability in the concentration of the working solution standard.

Diluent Selection: Since the method diluent is a mixture of ACN and aqueous buffer; ACN was selected as the diluent for the solution standard. The ampoule of Tadalafil stock solution in ACN can then be conveniently and accurately diluted before use in the analytical lab.

Preparation: While the method requires a small weight of reference material, the stock solution was prepared on multi-gram scale. Electrostatic controls and larger weights provide greater accuracy. Gravimetric addition of analyte and diluent eliminates issues with density and volume fluctuations with temperature.

Fill volume: 3 mL. Fill volume was selected for convenience of volumetric transfer and dilution of the stock at time of use.

Container: Unsilanized 5 mL wide-bore amber ampoules. Large enough to accommodate the volume required for dilution; wide bore allows use of volumetric pipettes. Flame sealed under argon – protects against oxidative and evaporative changes.

Baseline qualification: Potency and impurities by the validated method, assaying against the powdered reference material.

Stability: Short-term and long-term stability were evaluated by testing the solution for potency and impurities.

Development Solution – Proof of Concept

A solution was gravimetrically prepared for development purposes at 1 mg/mL in acetonitrile, a volume of 3 mL filled into 5 mL ampoules, and flame-sealed under argon.

This standard was subjected to short-term stress degradation conditions for 30 days. Control samples were stored in the sub-freezer. Stability samples were stored at sub-freezer, freezer, refrigerator, room temperature, 40°C, and 80°C conditions as well as photostability conditions and sampled at specified intervals.

Interval samples were transferred to the freezer and analyzed together at the end of the study. Potency and impurities were determined by the established methods.

Stress Degradation Plan

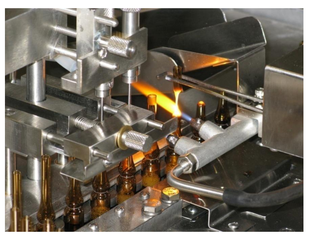
Temperature/ Condition	Timepoint (days)	Total # of Ampoules Stored	# Ampoules tested for each timepoint by:	
			Potency	Rel. Substances
80°C	3, 7, 14, 21, 30	36	3	3
40°C	7, 14, 30	24	3	3
15 to 30°C with simulated sunlight	Exposure up to 1.2 million lux hours	9 study 9 control	5 5	4 4
2 to 8°C	7, 14, 30	24	3	3
-80°C	7, 14	18	3	3
-25 to -10°C	Time 0	6	3	3

Stress Degradation Controlled Storage Assay Results

Timepoint (days)	Potency in mg/mL (RSD)				
	-25 to -10°C	-80°C	2 to 8°C	40°C	80°C
0	1.010	n/a	n/a	n/a	n/a
3	n/a	n/a	n/a	n/a	1.008
7	n/a	1.005	1.005	1.003	1.006
14	n/a	1.006	1.005	1.004	1.006
21	n/a	n/a	n/a	n/a	1.003
30	n/a	n/a	1.007	1.008	1.004

Stress Degradation Controlled Storage Impurities Results

Timepoint (days)	% Impurities				
	-25 to -10°C	-80°C	2 to 8°C	40°C	80°C
0	0.03%	n/a	n/a	n/a	n/a
3	n/a	n/a	n/a	n/a	0.03%
7	n/a	0.10%	0.05%	0.09%	0.05%
14	n/a	0.10%	0.05%	0.08%	0.07%
21	n/a	n/a	n/a	n/a	0.09%
30	n/a	n/a	0.07%	0.03%	0.10%

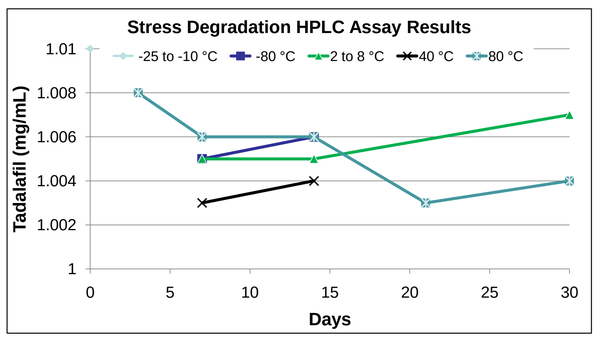


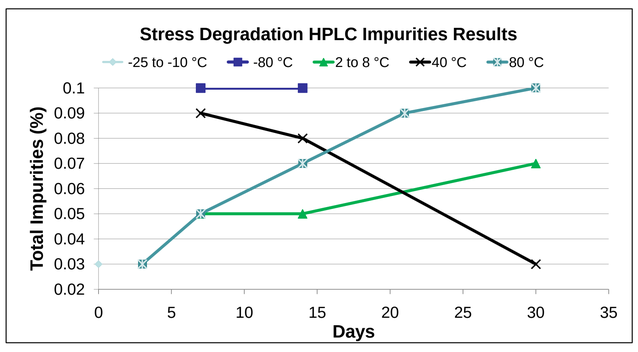
Stress Degradation Photostability Impurities Results

Exposure Conditions	% Impurities
Cerilliant (ICH 2)	0.06%
Cerilliant Control (ICH 2)	0.05%
Lilly Xenon (ICH 1)	0.15%
Lilly Xenon Control (ICH 1)	0.06%
Lilly Fluorescent (ICH 2)	0.10%
Lilly Fluorescent Control (ICH 2)	0.07%

Stress Degradation Photostability Assay Results

Exposure Conditions	% Impurities
Cerilliant (ICH 2)	1.005
Cerilliant Control (ICH 2)	1.005
Lilly Xenon (ICH 1)	1.000
Lilly Xenon Control (ICH 1)	1.003
Lilly Fluorescent (ICH 2)	1.007
Lilly Fluorescent Control (ICH 2)	1.006



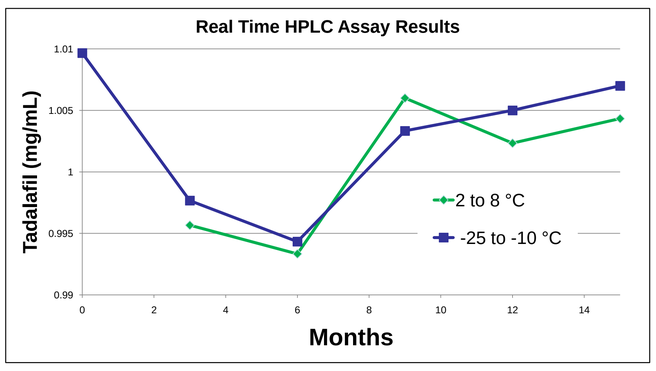


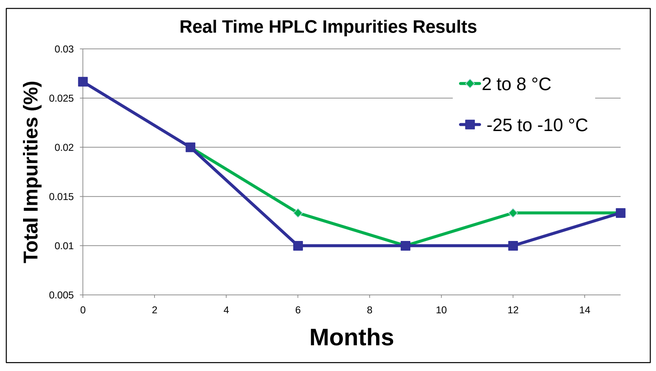
Potency was maintained under the conditions of the study. Photostability results indicated the solution was stable at normal laboratory use conditions. Long-term storage was assigned as refrigerated.

Long-Term Stability

The development solution was put on long-term stability under refrigerator and freezer conditions for two years. Neat reference material was used as assay standard in the potency measurement.

Stability Interval (months)	2°C to 8°C		-10°C to -25°C	
	Potency in mg/mL	Total Impurities	Potency in mg/mL	Total Impurities
15	1.004	0.01%	1.007	0.01%
12	1.002	0.01%	1.005	0.01%
9	1.006	0.01%	1.003	0.01%
6	0.993	0.01%	0.994	0.01%
3	0.996	0.02%	0.998	0.02%
Initial (t = 0)	NA		1.010	0.03%





Conclusion

A solution based assay standard was developed for Tadalafil. This standard replaced neat Tadalafil reference material for use in routine QC of Tadalafil.

The solution reference standard was prepared in a diluent compatible with the HPLC method and packaged under argon in flame-sealed amber ampoules.

The solution was stable under ambient handling conditions, stress degradation, and long-term up to 15 months to date. The assay standard prepared from neat, in contrast, has only been shown valid for two days.

The solution format eliminated documentation and storage requirements related to traceability of usage for special security substances. Time and efficiency in routine QC was improved by elimination of need for special procedures for handling of electrostatic materials during the weighing of assay standards. Day to day variability in assay standard preparation was reduced.

The study demonstrates that efficiencies can be gained by careful development of reference standards in formats that meet method requirements and also promote efficiency and consistency.

Data from 15 months of storage to date shows no loss of potency or increase in impurities.

Answers That Matter.

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