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DEVELOPMENT OF ACCELERATED STABILITY PROTOCOL FOR SILDENAFIL TABLETS – A EUROPEAN PERSPECTIVE REVIEW

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ABSTRACT

Frequently, the goal of a pharmaceutical company is to develop a globally acceptable registration stability protocol. A sound stability protocol not only eliminates unnecessary testing but also reduces manufacturing needs, cost and time. In this article considerable issues related to development of stability protocol such as type, size and number of batches, type, size and sources of containers and closures, container closures orientation, sampling plan, storage conditions, test time points, test parameters, test methods, acceptance criteria and the applicability of statistical methods for the analysis of stability data is discussed. The aim of this paper is to develop and outline accelerated stability protocol for Sildenafil tablets acceptable for registration in Europe and highlight some of the considerations that must be made before the execution of actual stability study.

KEYWORDS

Stability protocol; Sildenafil tablets; European Guidelines.

INTRODUCTION

The stability of pharmaceutical ingredients and the products containing them depends on two major factors:

- The chemical and physical properties of the materials concerned (including the excipients and container closure systems used for packaging of formulated products).
- Environmental factors, such as temperature, humidity and light and their effect on the drug products[1].

Frequently, the goal of a pharmaceutical company is to develop a globally acceptable registration stability protocol. A sound stability protocol not only eliminates unnecessary testing but also reduces manufacturing needs, cost and time. This is especially important in current scenario due to increase in the number of possible storage conditions and checkpoints because of stringent regional requirements[2].

The aim of this paper is to develop and outline important characteristics of a stability protocol acceptable for registration in Europe and highlight some of the considerations that must be made before the execution of actual stability study. Here the general requirements for development of stability protocol and stability testing of pharmaceuticals for

registration in the European Community (European Union) are discussed with respect to the sources of information on requirements and design of stability protocol for finished drug product[3].

The protocol contains an outline of the proposed plan to be used in generating stability data. The protocol describes the type of product being tested, sampling process, duration and frequency of testing, number of samples and replicates per time interval, storage conditions (length of storage, type of storage, temperatures and packaging), methods of analysis with associated supportive data, if available, and other tests[4].

The test protocol contains the following information

1. Type, size and number of batches.
2. Type of containers and closures, Pack detail and pack size
3. Sampling plan.
4. Test storage conditions.
5. Test time points.
6. Test parameters
7. Test methods
8. Acceptance criteria[5].

Although, all stability protocols contain a schedule for testing samples stored at one or more controlled storage conditions, the protocol can differ significantly from one product to another and from country to country. If a drug product is to be marketed solely in one country, then it is necessary to design a straightforward stability protocol on the basis of local regulations[6]. Although pharmaceutical companies follow the ICH guidelines for the stability testing but still there are some specific regional requirements of different countries which must be considered for stability studies and hence for getting approval of drug product. For example, for selection of batches ICH refers stability study to be carried out on at least three primary batches of the drug product, two of the three batches should be at least pilot scale batches and the third one can be smaller [7], however US recommends at least 3 lots of product of a typical batch size should be tested for stability[8] whereas according to European guidelines there are two options for selection of batches:

- For conventional dosage form, and when the active substance is known to be stable, stability study should be carried out on at least two pilot scale batches.
- For critical dosage form, and when the active substance is known to be unstable, stability study should be carried on at least three primary batches[9].

For containers and closures ICH & European guidelines refer stability study to be carried out on at least one container of each packaging material for testing where as US refers sampling of at least two containers of each packaging material for testing. FDA draft guidelines set the frequency as 0, 2, 4 and 6 months for 6 months accelerated stability data to be submitted along with the application. WHO & EU guidelines, however, suggest sampling at 0, 1, 2, 3 and 6 months under accelerated conditions. For the countries in zone I and II the storage conditions prescribed for long term testing are 25 ± 2ºC and 60 ± 5% RH (ICH and FDA) whereas WHO guidelines suggest that conditions for long term studies should be as close as to the derived conditions of 30 ± 2ºC and 35 ± 5% RH for zone III countries and 30 ± 2ºC and 70 ± 5% RH for zone IV countries[10]. Consideration of all these stringent requirements pointed out only one thing that it is utmost important to consider regional requirements while developing stability protocol. Hence, when we developed the accelerated stability protocol for sildenafil tablets for European market, we considered following parameters:

1. Type, size and number of batches

The guidelines emphasized the establishment of an expiration period that is based on limited number of batches. The testing of three batches provides a reliable estimate while testing fewer than three batches does not permit a reliable statistical estimate of batch to batch variability. Practical considerations prevent collection of broad amount of data because more data cause more stress on analytical facilities and increases the chances of errors. Testing fewer than three batches is allowed for stable and well established products. In European

guidelines for stability two options are available

Table 3.1 Pack details and Pack size

S. No.	Pack	No. of packs per station Strength(mg)			Total No. of packs per batch strength(mg)		
		25	50	100	25	50	100
1.	PVC blister (18tablets/blister)	40	30	25	640	480	400
2.	TRIPLEX blister (18tablets/blister)	40	30	25	640	480	400
3.	SBP pack 1200 tablets = 25mg 600 tablets = 50mg 300 tablets =100mg	2	2	2	6	6	6
PVC – Polyvinyl chloride **SBP Pack** – Simulated Bulk transfer Pack							

for selection of batches

- For the conventional dosage forms and when the active substances are known to be stable, stability data on at least two pilot scale batches is acceptable Or
- For the critical dosage forms and when the active substances are known to be unstable, stability data on three primary batches is to be provided. Two of three batches should be of at least pilot scale and third batch may be smaller.

As we are manufacturing sildenafil as a conventional dosage form (tablets) and it is known to be a stable molecule, therefore we have selected two batches of sildenafil of each strength, i.e., 25, 50 and 100 mg for conducting accelerated stability studies and the size of the batches for these different strengths are 200000 (tablets), 110000 (tablets), 105000 (tablets) for 25 mg, 50 mg and 100 mg strengths respectively.

2. ***Containers and closures, Pack details and pack size***

The stability protocol must contain the information about different types of containers and closures used in stability testing. The

protocol must provide information about the type, size and sources of containers and closures. The purpose of stability testing is not to qualify the container closure component labels, adhesives, colorants, inks, etc. but to determine the expiry period for the container closure combination as a whole. European guidelines suggest to conduct testing on samples enclosed in containers and closures proposed for storage and distribution. We selected three types of packaging material for Sildenafil tablets as shown in Table 3.1. We selected 40, 30 and 25 packs of PVC blister and TRIPLEX blister for strengths 25 mg, 50 mg and 100 mg respectively, required at each station and 2 simulated bulk transfer (SBP) packs for all strengths. Total number of packs required per batch are 640, 480, 400 packs of PVC and TRIPLEX blister for strengths 25 mg, 50 mg, 100 mg and 6 SBP for all strengths as required for 16 stations, i.e., one for initial testing, four for accelerated testing (1, 2 , 3 and 6 months), four for intermediate testing (3, 6, 9, 12 months) and seven for long term testing (3, 6, 9, 12, 18, 24, 36 months).

3. ***Sampling test time points***

According to European guidelines sampling frequency of every 3 months during the first year, every 6 months during the second year and then annually for drug products is suggested for long term testing whereas, testing frequency of 1, 2, 3 and 6 months is recommended for accelerated stability testing. So we have selected 1, 2, 3 and 6 months for accelerated testing of Sildenafil tablets.

4. ***Storage conditions***

Generally, a finished product should be evaluated under storage conditions that test its thermal stability and if necessary its sensitivity to moisture and potential for solvent loss. As per European guidelines storage conditions selected for sildenafil tablets are as shown in Table 3.2.

Table 3.2 Storage conditions according to European guidelines

Study	**Storage condition**	**Minimum time period covered by data at submission**
Long term	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH	6 months (option a) 12 months (option b)
Intermediate	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	months
Storage conditions for sildenafil tablets		
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

5. ***Test parameters***

The next step in stability protocol is to decide the test parameters. The parameters used for evaluation of the drawn sample vary with type of dosage form. In addition to drug assay and analysis of the degradation product(s) one should consider physical, chemical, biological and microbiological characteristics of the drug product(s) which are susceptible to change during storage. The properties specific to a particular dosage form should also be included in the test parameter list, including dissolution of the solid dosage form, because it can vary as product age[11].'

6. ***Setting specifications and acceptance criteria***

For different test parameters included in the stability study acceptance criteria should be fixed before performing stability study. The criteria can be set in the form of numerical limits if the result is quantitative (For example Moisture content, Assay, degradation product, particle size, microbial growth, etc.) or it can be descriptive if result is qualitative (For example Odour, colour, appearance, cracking) that can be expressed as pass or fail. According to European guidelines following tests are considered applicable to drug product (Sildenafil tablets) and corresponding acceptance limits are also set by considering these guidelines.

6.1 Description

This parameter involves description of the dosage form which is under consideration (e.g., size, shape, and color). If any of these characteristics change during manufacture or storage, this change should be investigated and corrective measure should be taken. The acceptance criteria should include the final acceptable appearance of the drug product.

Table 3.3 Test parameters for sildenafil tablets with Acceptance limits

S.No.	Test parameters	Acceptance limits	Evaluation time points (months)
1.	Description	Physical appearance should meet	0, 1, 2, 3, 6
2.	Identification	Should meet specification requirements	Initial
3.	Dissolution time	Drug release should be NLT 80% in 45 min	0, 1, 2, 3, 6
4.	Assay	(95 -105 %)	0, 1, 2, 3, 6
5.	Related substances -unknown impurity -Total impurity	 NMT 0.2%w/w NMT 0.4%w/w	0, 1, 2, 3, 6
6.	Disintegration time	NMT 15 min	Initial
7.	Average weight	For 25 mg = 154 ± 6mg For 50 mg = 309 ±12mg For 100 mg = 618 ± 24mg	Initial
8.	Content Uniformity	Should meet requirements	Initial
9.	Moisture content	NMT 6.0	0, 1, 2, 3, 6 months
10.	Microbial limits (TAMC) (TYMC) E.coli (per 1.0g)	 NMT 1000cfu/g NMT100cfu/g Must be absent	0, 6 months

NLT = Not Less Than
NMT = Not More Than
TAMC = Total Aerobic Microbial Count.
TYMC = Total Yeast Mould Count.
cfu = colony forming unit

6.2 Identification

Identification testing should set up the identity of the drug substance present in the drug product and should be able to distinguish between compounds of closely related structures. Identity tests should be specific for the drug substance, for e.g. infrared spectroscopy. Identification only by a single chromatographic procedure is not regarded as being specific. However, the use of two chromatographic procedures where the separation is based on different principles or combination of tests into a single procedure such as HPLC/UV, HPLC/MS or GC/MS is generally acceptable. We have selected HPLC/UV chromatographic procedures for identification test of sildenafil tablets.

6.3 Disintegration

For rapidly dissolving (dissolution >80% in 15 minutes at pH 1.2, 4.0 and 6.8) products containing drugs which are highly soluble throughout the physiological range, disintegration may be substituted for dissolution. Disintegration testing is most suitable when a relationship to dissolution has been recognized or when disintegration is shown to be more selective than dissolution[12].

6.5 Water content

A test for water content should be included when appropriate. The acceptance criteria may be justified with data on the effects of hydration or water absorption on the drug product. In some cases, a Loss on drying procedure may be considered adequate; however, a detection procedure which is specific for water (e.g. Karl Fischer titration) is preferred[14].

6.6 Microbial limits

Microbial testing is considered as an attribute of Good Manufacturing Practice as well as of quality assurance. Acceptance criteria should be set for the total count of aerobic microorganisms, the total count of yeasts and moulds, and the absence of specific objectionable bacteria (e.g. Staphylococcusbaureus, Escherichia coli, Salmonella, Pseudomonas aeruginosa). These should be determined by suitable procedures, preferably using Pharmacopoeial procedures at a time point in manufacture which is justified by data. The type of microbial test and acceptance criteria should be based on the nature of the drug substance, method of manufacture, and the intended use of the drug product[15].

6.7 Dissolution

The specification for solid oral dosage forms normally includes a test to measure release of drug substance from the drug product. By establishing appropriate test conditions and sampling procedures as official in pharmacopoeia single-point measurements are normally considered to be suitable for immediate-release dosage forms whereas multiple time point sampling should be performed for extended-release dosage forms, and two-stage testing may be appropriate for delayed-release dosage forms.

For immediate-release drug products where changes in dissolution rate have been established to significantly affect bioavailability, it is desirable to develop test conditions which can distinguish batches with unacceptable bioavailability.

For extended-release drug products, in vitro / in vivo correlation may be used to establish acceptance criteria when human bioavailability data are available for formulations exhibiting different release rates[16].

6.8 Impurities

Organic and inorganic impurities (degradation products) and residual solvents are included in this category.

Organic impurities arising from degradation of the drug substance and impurities that arise during the manufacturing process for the drug product should be monitored in the new drug product. Acceptance limits should be acknowledged for individual specified degradation products, which may include both identified and unidentified degradation products as appropriate and total degradation products. Process impurities arise from the drug substance synthesis are normally controlled during drug substance testing, and therefore not included in the total impurities limit. However, when a synthesis impurity is also a degradation product, its level should be monitored and included in the total degradation product impurity limit.

6.9 Assay

A specific stability-indicating assay to determine strength (content) should be included for all drug products. In many cases it is possible to take up the same procedure (e.g., HPLC) for both assay of the new drug substance and quantitation of impurities. Results of content uniformity testing for new drug products can be used for quantitation of drug product strength, if the methods used for content uniformity are also appropriate as assays[17].

Based on or after considering all these European regulations for stability testing we have set acceptance criteria and specification for sildenafil tablets. Test parameters and acceptance criteria selected for sildenafil tablets is as given in Table 3.3.

7. Test methods

For the test parameters that are included in the stability protocol, it is necessary to establish the test methodology. For the assay of the drug product it is advised to establish a stability indicating method, this method should be validated for specificity, accuracy, precision and linearity in the range in which drug is expected to fall during stability testing. Test procedures from European Pharmacopoeia are generally followed for performing tests included in stability protocol.

The next step after the stability study has completed is to analyze the data by applying appropriate statistical test like *Analysis of variance* (ANOVA). The *analysis of variance* (ANOVA) remains one of the most commonly used methods of statistical analysis in the statistical sciences[18]. *Analysis of variance* allows us to test the differences in the means of several different groups or populations. In order to test the hypothesis, an F statistic is calculated which compares the variation among the groups with the variation within the group[19]. By comparing the observed F value with the table value of F and then analyzing the significant difference we test our hypothesis.

CONCLUSION

Generally the success of a stability study depends upon the sound stability protocol. A successful stability protocol not only assures about our study but also reduces the chances of failure of study. When we developed the stability protocol for European market we observed that even after the harmonization of Stability Study guidelines there are some regional requirements which must be fulfilled in order to get the access to the market of interest. Therefore regional requirements should be emphasized during development of stability protocol. The purpose of considering regional requirements during protocol development is that this helps for registration for marketing in that region and this in turn indirectly results in higher level of reliability on the quality of the drug product.

REFERENCES

1. Carstensen, J.T and Rhodes, C.T. Drug stability principal and practices. Chapter 1.Introductory review.3rd edition revised and expanded. New York; Marcel Dekker, 2000
2. Beaman, J. Designing a globally acceptable registration stability protocol. *Pharmaceutical technology*. Mar 2007.
3. Carstensen, J.T and Rhodes, C.T. Drug stability principal and practices. Chapter 1.Introductory review.3rd edition revised and expanded. New York; Marcel Dekker, 2000
4. www.fda.gov accessed in 1997, Topic – Guidance for industry
5. Ali, J and Khar, R. K. A text book of dosage form design. Chapter 4. Stabilization of pharmaceutical products and stability testing, 1st Edn, Published by Birla publications Pvt. Ltd. New Delhi: 104, (2005).
6. Barron, M.D. Implementing successful stability testing operations. *Pharmaceutical technology Analytical chemistry & testing*, 2003; 14.
7. www.ich.org accessed in 2003. Topic - stability testing of new drug substances and products

8. www.fda.gov accessed in December 2008. Topic – guidance for industry drug stability guidelines
9. www.emea.eu.int accessed in 2000. Topic – stability testing of new drug substances and products.
10. www.who.org accessed in 2009. Topic - Stability testing of active pharmaceutical ingredient and finished pharmaceutical product.
11. www.emea.eu.int accessed in 2003. Topic – Guidance on stability testing: Stability testing of existing active substances and related finished products.
12. Pharmacopoeia European. Vol. I: Assays. Published by Directorate of medicinal and healthcare council of Europe, Germany: 2007, **6**; 278.
13. Pharmacopoeia European. Vol. I: Pharmaceutical technical procedures. Published by Directorate of medicinal and healthcare council of Europe, Germany: 2007, **6**; 278.
14. Pharmacopoeia European. Vol. I: Assays. Published by Directorate of medicinal and healthcare council of Europe, Germany: 2007, **6**; 263-264.
15. Pharmacopoeia European. Vol. I: Biological tests and general text on microbiology. Published by Directorate of medicinal and healthcare council of Europe, Germany: 2007, 6; 529-531.
16. Pharmacopoeia European. Vol.I: Pharmaceutical technical procedures. Published by Directorate of medicinal and healthcare council of Europe, Germany: 2007, **6**; 266-274.
17. www.emea.eu.int accessed in 2006. Topic – Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances.
18. Steiger, J.H. Beyond the f-test: effect size confidence intervals and tests of close fit in the analysis of variance and contrast analysis.
Physiological methods, **9(2):** 164-182, (1995).
19. www.visualstats.org accessed in 2000. Topic – Analysis of variance.