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Discussion on the Technical Requirements and Methods for In-use Stability Testing of Drug Products

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Abstract: This article introduces the concept, significance, and applicability of in-use stability testing for drug products. The article references relevant domestic and foreign guidelines for drug stability studies and discusses methods for evaluating the in-use stability of drug products based on the kinetic model of drug degradation.

1. Introduction

In-use stability testing refers to tests conducted to ensure the stability of a pharmaceutical preparation during a certain period of use [1]. While drug shelf life is based on conventional stability tests [2-4], for some drugs, such as those that need to be reconstituted during use or those packaged in multi-dose systems, it is also necessary to provide in-use stability test data. The environmental factors (temperature, humidity, and light), packaging, and long-term storage conditions of the in-use drug are different than those before use. The change of environmental factors during use and the destruction of the packaging's integrity increase the risk of instability, so domestic and foreign regulatory agencies require such drugs be subject to in-use stability studies. This article refers to the relevant guidelines of various countries on in-use stability, discusses the basic principles and concerns of the experimental design, and discusses methods for evaluating in-use drug stability based on kinetic models of drug degradation.

a. The importance of in-use stability testing of drug products

The main purpose of stability studies for in-use drug products is to determine the proper storage conditions during use and the effective period of use after opening, to ensure the safety of the drug once packaging has been opened. In order to ensure the drug's safe use, the test results should be included in the instructions, including the storage conditions and use period, the drug's compatibility with other drugs or diluents, the instability during use, and proper handling. For example, the storage section of the instructions for moxifloxacin hydrochloride eye drops says, "this product should be used within 28 days after opening and should be discarded after expiration."

b. Drug products requiring in-use stability testing

In-use stability studies are of great significance, but they are easily overlooked. Some categories of drugs require in-use stability studies, such as those needing to be reconstituted before clinical use, like powders for injections. These need to be dissolved and mixed with an appropriate amount of solvent (such as aqueous saline or glucose for injection) before use. Or small-volume, high-concentration injections, which need to be further diluted to larger-volume 5% glucose injections or saline injections before use. Part of oral solid preparations, such as replacement Grelor tablets, apixaban tablets, gefitinib tablets, etc., for patients who cannot swallow the whole tablet, can be crushed into a fine powder and suspended in water, 5% dextrose solution or apple juice, etc. before use orally in a vehicle or through a nasogastric tube. For such drugs, the product quality during the in-use period should be studied from the time of formulation to the end of use, because multiple factors in the formulation and use processes may cause changes in the key stability quality attributes of the drug. For example, the heat and physical force generated during grinding may cause the transformation of a drug's crystal form and accelerate the growth of degradant impurities. When a drug is dissolved or suspended in different solvents, various physical and chemical changes may occur. If the particle size of a drug becomes smaller after crushing, the area exposed to the solvent or the external environment will increase, which is more likely to accelerate degradation reactions. Low solubility drugs used for injection may precipitate during the preparation or storage of the drug product, resulting in a safety risk. The drug may also be contaminated by microorganisms and bacterial endotoxins during the preparation and in-use processes, which may then affect the sterility assurance level of the drug or increase its microbial load. According to "Guidelines for the Design and Research Technology of Functional Scoring of Oral Tablets for Chemical Generic Drugs (Trial)," for tablets with functional scoring, the stability of the divided part during actual use should also be examined [5]. Multi-dose packaged drugs, such as eye drops [6], oral solutions, ointments, gels, and some oral solid preparations, need to be opened and closed many times during use. Each time it is opened and used, environmental factors such as temperature, moisture, oxygen, light, microorganisms, and bacterial endotoxins will increase the adverse effects on the quality of the drug. In order to ensure the quality of the drug, stability studies for in-use drug products should also be carried out. Some drugs in high-barrier packaging, such as aluminum-plastic blisters and double-aluminum bag packaging, are usually used to protect from moisture and light. The double-aluminum bag is removed during use, so the packaging—moisture-proof and photo-protective before opening—no longer is as effective, increasing the risk of substandard drug quality. Therefore, in-use stability studies for such drug products should be carried out.

2. Regulatory requirements for in-use stability studies of drug products

a. Regulatory requirements of the Chinese National Medical Products Agency

Chapter 9001 of the 2020 edition of *Chinese Pharmacopoeia*, titled "Guiding Principles for Stability Testing of Raw Materials and Preparations," requires that some pharmaceutical preparations be prepared before use. The "Technical Guidelines for the Stability Research of Chemical Drugs (Raw Materials and Preparations)" clarifies that the relevant products for in-use drug stability studies are preparations that are made for temporary use, or those preparations with a certain period of use after the multi-dose packaging is opened. It points out that the long-term storage conditions and inspection time of the stability test should fully consider the entire process of storage and use [7]. At the same time, multiple guidelines such as the "Technical Guidelines for the Research on Pharmaceutical Changes of Listed Chemical Drugs (Trial)" [8] and the "Technical Guidelines for Pharmaceutical Changes During Clinical Trials of Innovative Drugs (Chemical Drugs) (Trial)" [9] have proposed some drugs also undergo in-use stability studies.

b. Regulatory requirements of the United States FDA

Chapter 797 of the General Principles of the United States Pharmacopeia stipulate that the in-use period for sterile preparations is the shortest use date based on factors such as product stability, sterility, and risk level [10]. Multi-dose-packaged sterile preparations, such as ophthalmic preparations (eye drops, ointments, aerosols, and other wound preparations) are susceptible to contamination by tears, wounds, and microorganisms in the environment during use and storage. Influencing the level of sterility protection, the in-use stability of drug products should be investigated.

c. European Medicines Agency (EMA) regulatory requirements

The EMA issued in 2001 a "Note for Guidance on In-Use Stability Testing of Human Medicinal Products," on the subject of in-use stability testing and the selection of batches, the experimental design, experimental conditions, experimental parameters, analysis process, data description, data evaluation, and manuals. This has provided a more detailed set of regulations [11].

d. Regulatory requirements of the International Conference on Harmonisation (ICH)

ICH Q1A (R) clearly states in the formulation section of "Stability Testing of New Drug Substances and Products," if necessary, how to test the preparation or dilution for stability. In-use stability testing provides the basis for labeling including storage conditions, and the use period after preparation or dilution. The declared batch should undergo a stability test both at

the time of formulation and the recommended shelf life after preparation and dilution as part of the formal stability test. If the test data of the entire shelf life cannot be provided before the declaration, the data of the 12th month or the most recent measurement should be provided [12].

e. Regulatory requirements of the World Health Organization (WHO)

The WHO updated the "Stability Testing of Active Pharmaceutical Ingredients and Finished Pharmaceutical Products" in 2018, which requires that the in-use stability testing of drug products simulate the actual in-use process as much as possible, with testing of physical, chemical, and microbiological characteristics during use. The test requires at least two batches of pilot-scale samples, and it is recommended that at least one of them be tested at the end of shelf life based on the long-term stability testing. If these data are not available, an in-use stability test needs to be carried out at the last stability time point of the registration application [13].

3. Test design and focus points of stability studies of in-use drug products

a. Basic principles of test design

a) Specificity. Basic principles should be followed when designing the trial and specific drug analysis should be done. The relevant domestic and foreign guidelines have clarified that the basic principle of in-use stability testing is to simulate the actual process of drug use as much as possible: that is, the environmental conditions under which it is placed, the form of packaging, the method of use, the number of users, the time of use, etc.

b) Covers the worst conditions of use. For example, an oral tablet in a multi-dose plastic bottle is stored under refrigeration and taken three times a day, one to two tablets each time. It is necessary to simulate opening and removing tablets at room temperature in the morning, afternoon, and evening each time. In order to cover the longest use time, one tablet should be taken at a time for 60 days, and samples can be taken at the beginning, and on the 15th, 30th, and 60th days for testing.

b. The selection of test samples based on the WHO, EMA, ICH, etc.

It is recommended to select samples at the end of shelf life for stability tests of long-term in-use drug products. This requirement has a scientific basis. For example, bottle packaging is the most common type of multi-dose packaging, and the mouth of the bottle is generally sealed by heat-sealing technology to improve the barrier effect of the bottle against water vapor. Studies have shown that even if the cap is tightened again after the bottle has been opened, due to the

lack of the effective barrier of the heat seal, the water vapor transmission coefficient is much higher than when heat-sealed [14]. When the bottle cap is opened and closed several times to take out the preparation, the water in the air outside the bottle will be in equilibrium with that in the bottle and the tablet. Therefore, during the use of bottled tablets, the relative humidity in the bottle may be significantly higher than that under long-term storage conditions. Even if a desiccant is added to the bottle, its effect of reducing the humidity in the bottle is limited because the drying effect is gradually weakened by the influence of humidity of the external environment as time extends during the shelf life. Especially when the environmental humidity is high, water in the air outside the bottle will penetrate the bottle even if the bottle's mouth is not open. Therefore, the stability of the drug used at different time points during the shelf life may vary.

Considering that, the test should simulate the worst conditions, and it is recommended to select products at the end of their shelf life for in-use stability studies. At the same time, for comparative analysis of test data, the ICH requires more sampling at the starting point of long-term stability than the WHO and EMA. In actual applications, due to limited stability data, test data at the end of the shelf life cannot necessarily be obtained; test data for 12 months or the last time point of long-term stability at the time of application and registration can then be provided. In this case, it is recommended to analyze the in-use stability data. If the stability data show a tendency to decrease, the in-use stability data at the end of the shelf life should be supplemented to support the rationality of the formulation's shelf life.

Regarding requirements for sample size and batches for in-use stability testing, the EMA and WHO require at least two batches, of which one batch is at least of pilot scale. The ICH does not specify batch quantity requirements but requires the use of the same master batch samples in the stability test. In view of the fact that the domestically declared stability samples should meet the pilot scale and above batch requirements [15], we also recommend using stability test samples for in-use stability studies, and that the batch quantity should be no less than two batches.

c. The design of the test plan

The environment in which the samples are placed during the test should be consistent with the storage conditions in the instructions. This product should remain stable within the validity period indicated on the label—e.g., "If stored at room temperature (~8–25°C), it should be used within two months." The storage conditions for the in-use stability test should be room

temperature, and the test duration should be long enough to cover the intended use period after preparation or multiple openings, in this case at least two months.

Consider that the storage conditions are not clear about the relative humidity of the environment (for example, the environmental humidity in different regions of my country is quite varied, the difference between the south and the north is great, and room temperature therefore fluctuates significantly). If the stability study shows that the drug is sensitive to temperature or humidity, in this case, consider extending the investigation time appropriately, or choose to conduct the in-use stability investigation under higher ambient temperature or humidity. The sampling time point of the test can be set by referring to the results of a routine stability investigation of the drug. If the stability of the drug is poor under high temperature or high humidity conditions, you should set as many sampling time points as possible; otherwise, the sample time points can be reduced. In principle, sampling is required. The time point is representative and, when a stability parameter changes significantly, it can be detected in time. The stability parameters are generally the same as those used with conventional stability testing. If necessary, special stability parameters should be set according to the quality requirements of different dosage forms (pyrogen or bacterial endotoxin, insoluble particles, color changes, etc.). Multi-dose packaged drugs containing antioxidants also need to be tested for the content of antioxidants during use to predict the trend of changes in antioxidant capacity during use and to provide a basis for the proposed period of use after opening the package.

At the same time, attention should be paid to the special impurities introduced during use. For example, for drugs that require solvent dilution before use, the specific degradation impurities caused by the solvent and the impurities that migrate into the drug-using containers such as syringes and nasogastric tubes should also be investigated. The analytical methods involved in the test research should be comprehensively validated, paying attention to whether the analytical method can meet the testing requirements of the in-use stability test. High method sensitivity and accuracy is required.

4. Evaluation of in-use stability based on the kinetic model of degradation

The aforementioned in-use stability test is a method recommended by domestic and foreign drug regulatory agencies, but it often faces two problems in implementation:

1) It is recommended to select samples at the end of the shelf life for testing, which often requires a long waiting time.

2) The stability of a drug product in actual use is affected by specific environmental temperatures, relative humidities and exposure times (for example, my country is generally hot and humid in summer and cold and dry in winter; the stability of preparations after exposure to different use environments is not the same, so it is difficult to obtain comprehensive data on in-use stability).

The above problems can be modeled through the kinetics of drug degradation. The kinetic model of drug degradation refers to a model that quantitatively relates the rate of drug degradation to environmental factors that drive degradation, such as temperature and humidity. Among them, the Accelerated Stability Assessment Program (ASAP) model is the most widely used: $\ln k = \ln A - E_a /RT + B (RH)$. In this model, k is the ratio of the specification limit to the isoconversion time (time to hit the specification limit); A is the pre-factor, also known as the Arrhenius constant; E_a is the activation energy; T is the absolute temperature; R is the molar gas constant; B is a humidity-sensitivity factor describing how a degradation rate is affected by humidity; and RH is the equilibrium relative humidity. Compared with the classic Arrhenius equation, this formula has two improvements: the equation includes a moisture sensitivity factor item of B (RH), and the equation is expressed in terms of the ratio of the specification limit to the isoconversion time (that is, the ratio of the specification limit to the time required to reach the specification limit) rather than the reaction rate (the change in impurity or content per unit of time). These two improvements have increased the scope of application of the ASAP model and improved the accuracy of predictions, which is of great significance. Among them, the addition of B (RH) water-sensitive factors can reflect the influence of relative humidity on the equivalent conversion time; the replacement of k can effectively solve the nonlinear drug degradation kinetics type that cannot be solved by the classic Arrhenius kinetics (accounting for more than 50% of stability parameters in drug products). The ASAP model has been proven to have sufficient accuracy in a number of implementation cases to accurately predict the shelf life and in-use life of products [16].

The establishment of the ASAP degradation kinetic model requires four steps. First, the sample is exposed to high temperatures and high humidity conditions in an accelerated test for two to four weeks. Second, liquid chromatography or other quantitative methods are used to analyze the sample. Third, the conversion time is calculated under different acceleration conditions to solve the kinetic model parameters. And finally, after obtaining the kinetic parameters, you can specify any key parameters such as ambient temperature, relative humidity, and usage times for comprehensive system in-use stability assessment and prediction. This method can

effectively shorten the test duration and, at the same time, investigate in parallel the drug product's in-use stability under multiple temperatures and humidity environments.

However, there are also some limitations. The parameters ln A and E_a in the formula determine the sensitivity of the degradation reaction to temperature, and B (RH) determines the sensitivity of the degradation reaction to humidity, but the formula does not take into account the influence of light on the degradation reaction. The range of actual in-use conditions for drug products is also complex and difficult to specify to use in the calculations.

The advantage of in-use stability evaluation based on this kinetic model of drug degradation is that this method can be used as a supplement to the in-use stability test, providing data support for the subsequent formulation of the in-use stability protocol, and is especially

5. Conclusions

The stability of a drug during use is related to its safety and efficacy. Therefore, a systematic study is needed to provide a basis for the proposed storage conditions and in-use period. One should usually refer to domestic and relevant guidelines such as those from the WHO, EMA, ICH, etc. for in-use stability testing procedures. For drugs with less long-term stability data at the time of declaration, especially if it is a new drug, much can be learned about the environment's effects on that drug by establishing a degradation kinetic model. The influence of factors such as temperature and relative humidity are quantified to achieve a shorter test duration. Fewer test resources are used within the time limit, and the in-use stability risk can be evaluated. Both traditional stability testing methods and those using a kinetic model of degradation have their own advantages and disadvantages, and they should be taken into consideration with the dosage form when choosing features, environmental factors that accelerate the degradation reaction, packaging form, and usage times. Comprehensive consideration of both methods' data can be used in combination with each other to improve results. The process for determining in-use stability for a drug product must meet regulatory requirements while still being efficient.

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