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Stability and compatibility study of parenteral diazepam in different storage conditions

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ABSTRACT

Diazepam injection was subjected to different stress conditions, as prescribed in ICH guidelines with an emphasis to both pristine drug as well as final formulation. The degradation of diazepam was found to occur mainly in hydrolytic and to some extent in photolytic conditions, while the drug was stable to oxidative and thermal stress. The drug was particularly labile under neutral and alkaline hydrolytic conditions. Diazepam sample was exposed to accelerated, thermal and photodegradation conditions as observed during hot and humid summer conditions in Indian subcontinent as per ICH guidelines. The samples were analyzed by a validated high performance liquid chromatographic method. Results obtained confirmed the photo and hydrolytic labile nature of diazepam. Thus, an appropriate protection is recommended during storage and handling of this tranquilizer such that the assay, purity and potency of the drug is maintained.

Keywords: Diazepam injection, stress conditions, photostability, ICH guidelines, HPLC.

INTRODUCTION

Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of drug product. The FDA and ICH guidances state that there is a requirement of stability testing data to understand how the quality of a drug substance and drug product changes with time under the influence of various environmental factors [1-4]. Knowledge of the stability of molecule helps in selecting proper formulation and package as well as providing proper storage conditions to achieve the desired shelf life, which is essential for regulatory documentation. It also ensures prescribed standards of physical, chemical, therapeutic and toxicological specifications to ensure the identity, strength, quality and purity of a formulation. However, the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light as well as on the product related factors e.g chemical and physical properties of active substance and of pharmaceutical excipient, the dosage form and its composition, the manufacturing process, the nature of the container closure system and properties of packaging material [5-6]. Thus, the stability study is essential not only for regulatory approval but also ensures safety of the patient.

Diazepam is a benzodiazepine derivative chemically designated as 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (Figure 1).

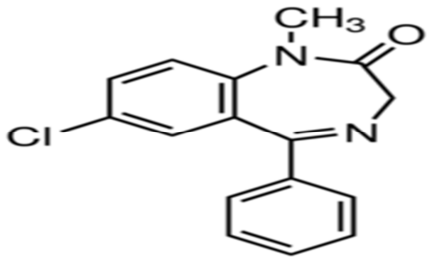

Figure 1: Chemical structure of dizepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one)

It is the most frequently prescribed benzodiazepine used in the treatment of anxiety, insomnia, seizures and alcohol withdrawal. It binds to a specific subunit on the GABA receptor at a site that is distinct from the binding site of the endogenous GABA molecule. Diazepam appears to act on areas of the limbic system, thalamus and hypothalamus, inducing anxiolytic effects. Its actions are due to the enhancement of GABA activity [7-8]. Diazepam is very slightly soluble in water, soluble in alcohol, and freely soluble in chloroform. In aqueous solution, diazepam is relatively stable at pH 4 - 8 with maximum stability at about pH 5. Stability and solubility is increased in mixed solvent systems of water, ethanol and propylene glycol [9]. The solution for parenteral injection should be protected from light and freezing. Diazepam can absorb into plastics, so liquid preparations should not be kept in plastic bottles or syringes, etc. [10]. As such, it can leach into the plastic bags and tubing used for intravenous infusions. Absorption appears to depend on several factors, such as temperature, concentration, flow rates, and tube length. Diazepam should not be administered if a precipitate has formed and does not dissolve.

The stability of diazepam parenteral formulation under various stress conditions were reported elsewhere in the literature. The stability of diazepam (5 mg/mL) injectable as a function of storage temperature was evaluated at three storage conditions: 4°C to 10°C (refrigerated); 15°C to 30°C and 37°C (oven-heated) and these injections were stored for 210 days in clear glass syringes. HPLC analyses of syringe contents were performed at 30-day intervals. After 210 days, the reduction in diazepam concentration was 7% refrigerated, 15% at ambient temperature, and 25% at 37°C. These results suggested that diazepam can be stored at room temperature. When drug storage temperatures exceed 30°C, more frequent stocking or refrigeration is required [11].

In another study, stability of diazepam injection repackaged in disposable glass syringes and stored at room and refrigerator temperatures was studied. The syringes were stored in light-resistant bags on their sides so that the solution was in contact with the rubber stoppers on both ends. Samples were assayed with a stability-indicating HPLC method for diazepam and its degradation product, 2-methylamino-5-chloro-benzophenone (MACB), after 30, 60, and 90 days of storage at 30 ºC and 4 ºC. After 90 days, the diazepam injection stored at 4 ºC retained 97.4 +/- 1.6% of its original potency; samples stored at 30 ºC retained 92.4 +/- 1% of their original potency. Apparent absorption to rubber syringe components led to a decrease in the concentration of both diazepam and MACB. The decrease in diazepam was a function of storage time and temperature; however, the disappearance of MACB from the syringes was a function of only time. The findings pointed towards stability of diazepam injection presented in the disposable glass syringes of diazepam can be stored for 90 days when stored at 4 ºC or 30 ºC [12]. The compatibility and stability of diazepam injection were also studied following dilution to 10 different concentrations in 5% dextrose, normal saline, Ringer's injection and lactated Ringer's injection. Prepared solutions were examined for clarity and pH throughout a 24-hour period. The study revealed that diazepam injection is stable in 5% dextrose, normal saline, Ringer's injection or lactated Ringer's injection to a dilution of at least 1:40 and used within 6 hours or to a dilution of 1:50 and used within 24 hours [13]. Further, long term stability study also claimed less than 1.5% degradation of diazepam was even after ten years [14].

The parenteral products in solution form needs to be tested for its assay and related substance to ensure its purity, safety and accuracy when used in a clinical setting. The pH, temperature, light and oxidation are the critical environmental factors for drug product degradation. The aim of this study was to investigate the stability and compatibility of Diazepam active pharmaceutical ingredient (API) and its finished formulation as injectable at different storage conditions as per ICH guidelines and to evaluate the drug product degradation to ensure its potency and safety.

EXPERIMENTAL SECTION

2.1 Materials

An analytically pure sample of diazepam was procured from Sigma Aldrich, Germany. Ultra pure water, acetonitrile and methanol (HPLC grade) were procured from Merck, Germany. Potassium dihydrogen phosphate (AR grade,

purity 99.6%) was procured from Merck, Germany. Diazepam injection was procured from a local pharmacy with labeled amount 5mg/ml. Other chemicals used were of analytical grade and used as received.

2.2. Instrumentation and chromatographic conditions

Chromatographic separation was performed as per the method reported in USP 32 with minor modification using an Agilent 1200 series liquid chromatographic system equipped with G1311A quaternary pump, Agilent variable-wavelength UV-Vis detector, and a G1329A auto injector. EZ CHROME ELITE software was employed for data collecting and processing. Samples were centrifuged by using a Remi Centrifuge (C-24BL).

The mobile phase consisting of a binary mixture of methanol and water (65:35), was filtered through 0.45 µm membrane nylon filter (Millipore), degassed prior to use. The flow rate of 1.4 mL/min. Prepare a solution of p-tolualdehyde in methanol containing about 0.3 µL/mL and used as internal standard. The liquid chromatograph was equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate was taken about 1.4 mL/minute.

2.3 Preparation of Solutions

2.3.1 Preparation of reference solution

The reference solution was prepared by dissolving an accurately weighed quantity of USP Diazepam RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 1 mg/mL. 5.0 mL of this solution was added to a 25 mL volumetric flask, then 5.0 mL of Internal standard solution added, diluted with methanol to volume, and mixed to obtain a standard preparation having a known concentration of about 0.2 mg of USP Diazepam RS/mL.

2.3.2 Preparation of test solution

The test solution were prepared by transferring an accurately measured volume of Injection, equivalent to about 10 mg of diazepam, to a 50 mL volumetric flask. 10 mL of Internal standard solution was added to the flask, diluted with methanol to volume, and mixed properly.

2.4. Physical characterization

The Diazepam pure drug and commercial samples were evaluated for visual observation, identification, pH and assay as per the method reported in Indian pharmacopoeia 2014 and USP 32.

2.5. Stability studies

Stability studies were carried out at different temperature and humidity conditions as per ICH guidelines, viz. 25ºC ± 2ºC/60% RH ± 5% RH, 30ºC ± 2ºC/65% RH ± 5%RH, 40ºC ± 2ºC/75% RH ± 5% RH. The tests were carried out as per ICH guidelines as suggested from time to time.

2.6 Stress degradation of diazepam

For stress degradation studies, diazepam pure drug and diazepam injection were subjected to thermal and photodegradation studies. The stress studies were carried out under the conditions as defined by ICH [16-18].

2.6.1 Thermal degradation studies

Thermal degradation studies were performed using a dry air oven. Susceptibility of the diazepam and diazepam injection to dry heat was studied by exposing at 50 °C for seven days (25 degree celsius of air temperature corresponds to 50 degree celsius goods temperature inside brown painted cargo container) [19]. At different time intervals, amounts of diazepam samples (Three ampoules for each time) were taken and diluted in sterile distilled water to give final concentration of diazepam as reported in section 2.3.2 and evaluated using validated HPLC method.

2.6.2 Photodegradation studies

Photodegradation studies were carried out in a photostability chamber. For photo-stability testing, a cool, white, fluorescent lamp designed to produce an output similar to that specified in ISO 10977 (1993) and a near-UV fluorescent lamp having a spectral distribution from 320 to 400 nm (with maximum energy emission between 350 and 370 nm; a significant proportion of UV in both bands of 320-360 nm and 360-400 nm) of ICH Q1B (Option-2) were used in the photo-stability chamber (Model no: TP 200S) manufactured by Thermolab Scientific Equipments (Thane, Maharashtra, India)[24]. Samples were exposed to the cool white fluorescent light providing overall illumination of not less than 1.2 million lux hours and an integrated near-UV energy of not less than 200 W h/m^2 [20]. The controls of temperature and relative humidity were maintained appropriately constant throughout within 30 ± 2°C and 65 ± 5% relative humidity, respectively, to minimize the effect of localized temperature and humidity changes. The diazepam pure drug and diazepam injections were exposed to ultraviolet radiation and visible

radiation for 1, 7, 15, 21 and 28 days. At the predefined time intervals, aliquots were withdrawn at suitable time intervals (Three ampoules for each time point) and diluted in sterile distilled water to give final concentration as reported in section 2.3.2.

RESULTS AND DISCUSSION

The study of photo, thermal and chemical degradation in the stability of drugs is one of the most concerned areas in the field of drug development and formulation. Stability of a pharmaceutical product may be defined as the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, toxicological, protective and informational specifications [21-22]. In other words, it is the extent to which a product retains, within the specified limits, throughout its period of storage and use, the same properties and characteristics possessed at the time of its packaging. Stability testing thus evaluates the effect of environmental factors on the quality of the a drug substance or a formulated product which is utilized for prediction of its shelf life, determine proper storage conditions and suggest labeling instructions. Moreover, the data generated during the stability testing is an important requirement for regulatory approval of any drug or formulation [23]. In this study, thermal and photostability studies of diazepam were carried out through employment of stress conditions. Thermal degradation profile of diazepam was studied at both 40 ºC and 50 ºC for different time periods and the photodegradation of reconstitution product was studied for different time periods to mimic the possible environmental conditions as suggested by ICH guidelines and the temperatures which are prevalent in Indian subcontinent during summer seasons with a particular reference to hot and humid conditions.

3.1 Development of analytical method

The relative retention times are about 0.5 for p-tolualdehyde and 1.0 for diazepam, the tailing factor for the diazepam peak is not more than 2.5, the resolution, R, between the p-tolualdehyde and diazepam peaks is not less than 3.5, and the relative standard deviation for replicate injections is not more than 2.0%. The method seems to be suitable for analysis of drug during stress degradation studies.

Table 1: Physical characterization of diazepam pure drug

Test	Results	Inference
Description	Light yellow	Meets IP 2014
Identification	IR spectrum	Meets IP 2014/USP 32 Standards
PH (1% w/v in WFI)	6.52±0.24	Meets IP2014/USP 32 standards
Assay	100.7±0.14	Meets IP 2014/USP 32 standards

Table 2: Physical characterization of diazepam injection

Test	Results	Inference
Description	Light yellow	Meets USP 32 standards; Fails IP 2014 Standards
Identification	Absorption maxima at about 242 and at about 284	Meets IP 2014/USP 32 Standards
	Retention time of the major peak in the chromatogram of the test Assay preparation corresponds to that of the Standard preparation in assay test.	Meets IP2014/USP 32 standards
pH	6.41±0.19	Meets IP 2014/USP 32 Standards
Assay	99.73±1.57	Meets IP2014/USP 32 standards

3.2 Physical characterization

Table 1 and table 2 showed the results of physical characterization of diazepam pure drug and diazepam injection respectively. Diazepam, an almost white to pale yellow powder had a pH of 6.5±0.24 and assay was found to be 100.7±0.14. Diazepam injection identified both by IP2014 and USP 32 method meets the standards. The pH (6.41±0.19) and assay (99.73±1.57) of diazepam injection were found well within limits. Thus, the physical parameters stayed practically unchanged during three months of storage of both diazepam and diazepam injection. However, diazepam injection seems transparent to light yellow in colour and so fails in the description part of Indian pharmacopcoeial specifications.The description in colour of the injection is confounding as it can be noted that the pristine diazepam API is also yellowish in colour. Thus, the clear to pale yellow colour of the finished product as specified in the USP32 is more achievable/justifiable as described in the literature and in view of the light sensitivity of diazepam API and finished product. The reversible molecular rearrangement reaction from a seven member ring to a six member ring and vice versa leads to the possible colour change which does not jeopardize the patient safety

and thus the description in USP 32 for the finished product is more suitable. To ensure the safety aspects of diazepam injection, further stability studies were conducted.

3.3 Stability study

This study is important to identify what attribute was susceptible to change during storage, as well as the level at which that quality attribute remain within acceptance criteria. The stability study of diazepam pure drug and diazepam injection under intermediate conditions and accelerated stability conditions showed no precipitation and change in pH and color change were observed in any of the samples during the three months of storage in both storage conditions (At 30°C±2°C/65%RH±5%RH and 40°C±2°C/75%RH±5%RH). The percent diazepam remaining in the diazepam and its parenteral formulation over a 3 month period was greater than 90%. No difference was observed between formulations and storage conditions (Table 3). The average pH of the samples (between 6.2 and 6.83) was able to minimize the degradation of diazepam in its parenteral formulation.

3.4 Accelerated testing

In case of accelerated stability studies, the rate of chemical reaction is increased by increment in temperature which ultimately increases the rate of chemical degradation or physical change of a drug substance or drug product by utilizing exaggerated storage conditions. In addition to long term studies, the chemical effects at non accelerated conditions and the effect of short-term excursions as might occur during shipping can be assessed simultaneously. The accelerated testing studies' results may always not be predictive of physical changes (Guidance for Industry Q1A(R2) Stability Testing of New Drug Substances and Products). The elevated temperatures are mainly used in accelerated testing which may increase the rate of reactions, but the outcome may be different in case of a cold chain product. It is useful to estimate the rate of reaction at single or different temperature and extrapolation to the desired temperature, thereby predicting the ambient stability [24]. The thermolabile products further deteriorate due to absorption of thermal energy which decrease the assay of active ingredient or depreciate physical appearance such as coloration of some components, modification of dissolution rate or cracking / separation of emulsions.

3.4.1 Thermal degradation studies

Temperature has a high degree of influence on all varieties of chemical reactions and it is usually accelerated by raising the temperature. This is understandable since with increased temperature, molecules tend to move faster with increased kinetic energy. Additionally, the rate of collision molecules increases greatly. Finally, greater available energy causes more molecules to have enough activation energy and the fraction of collisions with suitable energy increases. It is typically said that a 10 °C increase in temperature produces a 2-5 fold increase in decomposition [25]. Thus, thermal degradation studies are important as these studies provide evidence on how the quality of API or finished product varies in different storage conditions. When the diazepam and diazepam injection were exposed to 50 °C showed less than 5 % degradation in seven days (Table 3). Thus, diazepam is a thermally stable drug. Similar findings were also observed with diazepam injection.

3.4.2 Photodegradation studies

The active principles, product formulation and packaging can be affected by light. There may be bleaching of colored compounds or a possible discoloration of colorless / white coloured products. The loss of potency of the product is the most common degradation effect as observed with pharmaceuticals. This has further consequence of rendering the drug therapeutically inactive. In addition to this, photodegradation products formed in the pharmaceutical preparation may lead to adverse effects which is concentration dependent [26].

It is essential to possess basic details regarding photoreactivity of compounds to furnish information pertaining to handling, packaging, labeling and use of the drug substance or drug product. The mechanistic knowledge about photodegradation is of importance in stabilizing the product. Exposure to electro magnetic radiation and the transfer of radiant energy to the substance may directly or indirectly affect the drug molecules. As a matter, the transfer and uptake of energy in the form of radiant light subsequently degrades the active substance [25, 27-29]. The ultraviolet irradiation present in sunlight along with visible spectra as well as artificial light sources such as fluorescent light mainly contribute to the photodegradation of the active substance which alters the physicochemical properties of the product, e.g. discoloration or cloudy appearance, viscosity loss, change in dissolution rate or precipitation etc. [25].

Among the factors influencing drug degradation the photostability or photosensibility of pharmaceuticals is an area of growing concern as the number of drugs found to be photosensitive is increasing. In this study, photostability studies of diazepam were carried out through employment of stress conditions. Photodegradation was studied at both ultraviolet and visible radiation also for different time periods. In this study, thermal and photostability studies of diazepam were carried out under stress testing conditions. The thermal degradation profile of diazepam was studied at both 40ºC and 50ºC and the photodegradation of pristine sample and finished formulation was studied for different time periods to mimic the possible environmental conditions as suggested by ICH guidelines and the

temperatures which are prevalent in Indian subcontinent during summer seasons with a particular reference to hot and humid conditions. In this work, experiments were carried out using commercial samples, in sealed glass vials, protected from humidity. On exposures of diazepam to ultraviolet and visible radiation in liquid state, this drug was found to be stable. HPLC results also show that the retention time and peak area of diazepam remains unchanged and no significant degradation was observed upon photo exposure within the indicated period, suggesting that diazepam is photostable even after 21 days (Table 4).

Table 3: Stability study results of Diazepam pure drug and diazepam injection exposed at 30°C±2°C/65%RH±5%RH, 40°C±2°C/75%RH±5%RH and at 50°C

Period (months)		Stability conditions for diazeapm (Assay:Not less than 95.0 percent and not more than 105.0 percent of $C_{16}H_{13}ClN_2O$)and diazepam injection (Assay:not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{16}H_{13}ClN_2O$)					
		30°C±2°C/65%RH±5%RH		40°C±2°C/75%RH±5%RH		at 50°C	
		Diazepam API	Diazepam injection	Diazepam API	Diazepam injection	Diazepam API	Diazepam injection
Initial	Assay	100.7±0.14	99.73±1.57	100.7±0.14	99.73±1.57	100.7±0.14	99.73±1.57
	Description	Light yellow	Pale yellow	Light yellow	Pale yellow	light yellow	Pale yellow
After 1st month	Assay	97.34±0.47	96.54±1.24	95.49±1.35	94.65±1.19	97.48±0.49 (After 1 day)	97.75±1.05 (After 1 day)
	Description	Light yellow	Pale yellow	Light yellow	Pale yellow	Light yellow	Pale yellow
After 3rdmonth	Assay	93.49±0.94	91.88±0.86	92.87±1.14	91.79±0.26	95.48±0.98 (After 7 day)	95.19±1.14 (After 7 day)
	Description	Light yellow	Pale yellow	Light yellow	Pale yellow	Light yellow	
After 6th month	Assay	88.98±1.67	NP*				
	Description	Mild yellow	Mild yellow				

NP: Not performed

Table 4: Photostability studies results of the determination of diazepam and diazepam injection after exposing to photostability chamber as per ICH guidelines

Period (days)	Photostability of diazeapm (Assay:Not less than 95.0 percent and not more than 105.0 percent of $C_{16}H_{13}ClN_2O$)and diazepam injection (Assay:not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{16}H_{13}ClN_2O$)			
	Diazepam API		Diazepam injection	
	Assay	Degradation (%)	Assay	Degradation (%)
Initial	100.7±0.14	0%	99.73±1.57	0%
After 1st Day	99.56±0.49	1.14%	97.98±0.84	1.75%
After 2nd Day	98.11±0.88	2.59%	96.39±0.79	3.34%
After 7th Day	95.19±0.77	5.51%	94.39±0.69	5.84%
After 14th Day	93.18±0.79	7.52%	93.54±0.76	6.19%
After 21st Day	92.34±0.68	7.73%	91.49±0.64	8.24%

A higher degradation in daizepam injection as compared to diazepam was observed. However, it was within the limits of pharmacopoeial specifications. A yellowish color developed thereupon exposures of diazepam injection to heat and ultraviolet radiation. However, other parameters such as assay remains within limits and such color changes may be acceptable. From the results of the photodegradation studies, it was observed that the drug in the solid state was comparatively more photostable than in solution. Further exposure is required to determine the photodegradation of diazepam or diazepam injection.

CONCLUSION

In the present research work, a systematic approach was successfully applied to better explore the stability of diazepam and diazepam injection in different environmental conditions and comparison with pharmacopoeial specifications.

In view of the above findings, it is suitable to store the diazepam injection below 30 °C, protected from light. The slight pale yellowish colour as suggested by USP may be quantified in the description and adopted in other pharmacopoeias also to prevent any ambiguity in the description of the finished product for harmonization. Consequently, an appropriate protection is recommended during storage, handling and reconstitution of the product. Thus, the product degradation can be minimized and the pharmaceutical safety and efficacy can be maintained. A further, risk based study is required for pharmaceutical development to ensure the product safety, integrity and quality for patient.

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