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Submission date: 30-Sep-2021 11:20PM (UTC+0800)
Submission ID: 1661629444
File name: 11624-Article_Text-22187-midazolam_1.pdf (730.37K)
Word count: 3188
Character count: 17770

Comparative Test of Midazolam Hydrochloride Stability in Different Storage and Temperature Container

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Abstract

Background: Midazolam hydrochloride is an injection form of benzodiazepines and included in the high alert category in the Dr. Soetomo Teaching Hospital Intensive Care Unit (ICU), Surabaya, Indonesia. Nowadays, the technical preparation of midazolam drugs in the ICU room by doctors and nurses was performed by reconstituting drugs in the injection syringes to be stored for a while in the room. **Objectives**: To evaluate the preparation process for midazolam hydrochloride injection which has been analyzed in Dr. Soetomo Teaching Hospital. **Methods**: Physical, chemical, and microbiological stability of 1 mg/mL midazolam hydrochloride in aqua pro injection solvents have been evaluated at points 0, 8, 12, and 24 hours after preparation, as well as a comparison of stability in storage conditions at room temperature compared to refrigerator temperatures. **Results**: The results showed all the preparations were no foreign particles in the sample container. The midazolam did not experience precipitation in both room and refrigerator temperatures. The pH of midazolam solution during storage proved that the preparation was relatively stable (pH 4.3–4.5). There were no significant differences of midazolam levels in both storages which was in range of 95%. The Miccrobiology Stability Test showed negative germ growth after 24 hours incubation in both storage. **Conclusion**: This study showed no changes in physical, chemical, or microbiological stability in the midazolam injection samples up to 24 hours after the manufacturing process.

Keywords: *Midazolam Hydrochloride, Injection Preparation, Aseptic Technique, Stability Test*

Introduction

The stability of pharmaceutical dosage forms is strongly influenced by chemical, physical, and microbiological reactions. These reactions can lead the changes of medicinal properties during the process of shipping, storage, and use. Chemical degradation is known as the cause of a potential decrease or increase in drug toxicity. The storage condition also cause dissolution and pollination process of other medicinal ingredients, so that affect to the stability of the drug[1].

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Midazolam hydrochloride is an injection form of benzodiazepines with a short reaction period. Intramuscular route is often used for midazolam administration, then continuous to iv infusion and iv slow bolus[2]. Intramuscular midazolam administration can terminate acute seizure in children besides intranasal midazolam[3,4]. Previous research showed the stability of midazolam is around 24 hours in normal saline 0.9% or Dextrose 5%[5,6]. Technical midazolam preparation is usually carried out by the drug reconstitution of injection syringes preparation and drugs storage in the room. It aims to facilitate the administration in emergency condition, which needs immediate administration[7,8].

Patient safety becomes priority in the management of drug use (MDU). The policy of MDU, namely standard operational procedure for mixing injectable

medicines, explains that the single component does not recommend storing reconstitution drugs in injection syringes, but in sterile vial containers covered by film paper[9]. The reconstitution drugs do not stored for more than 8 hours[9]. However, the MDU policy on injecting drug reconstitution is not supported by accurate data from preliminary research results both qualitative and quantitative aspects.

Baseline reference for the MDU policy for the reconstruction of single preparations is existed in Dr. Soetomo Teaching Hospital. This preliminary study determined the qualitative and quantitative stability of 1 mg/mL midazolam injection in aqua pro injection solvents, whether the stability of the drug was more than 8 hours or 24 hours. The stability test compared to the parameters of the storage container will be observed from the room temperature. The room temperature was 20-25 °C, compared to the refrigerator temperature (2-8 °C). The qualitative stability measured by physical, chemical, and microbiological stability. However, quantitative stability is conducted by determining levels using High-Performance Liquid Chromatography (HPLC) tools. The results of this study can be a reference data for the guidelines evaluation for injecting drug mixing policy in Dr. Soetomo Teaching Hospital. The purpose of this study determined by using qualitative and quantitative stability of midazolam reconstitution HCl 1 mg/mL with storage container parameters.

Methods

Material

Raw midazolam hydrochloride was a gift from Kalbe Farma Factory. Acetonitrile and potassium hydrogen phosphate were Wako products (Osaka, Japan). Sterile glass vials and injection syringes (Terumo®, Tokyo, Japan) were purchased from the Medical Pharmacy Service Unit, Dr. Soetomo Teaching Hospital, Surabaya, Indonesia. Other research materials were analytical grade.

Test Solution Preparation

Preparation of the test solution sample was examined by the same possible conditions that was conducted by the nurse or doctor when reconstituting midazolam hydrochloride in the ICU inpatient ward of Dr. Soetomo Teaching Hospital. Two midazolam hydrochloride test solutions of 1 mg/mL were prepared for replication three times per test procedure. The first solution was prepared in a Terumo® injection syringe container (containing polypropylene material) and a second solution was prepared in a sterile vial storage container covered with parafilm paper. The first mixing container (5 mL injection syringe, Terumo®) was filled by midazolam hydrochloride 1 mg/mL. The concentration was obtained by mixing technically as follows: A total of 1 ampule of midazolam hydrochloride 5 mg/mL was diluted and a number of 20 mL aqua pro injection to obtain a 1 mg/mL level test solution. The solution was taken 1 mL to be stored in a 5 mL injection syringe. The second solution was prepared in a sterile vial storage container with a dilution procedure: 1 ampoule midazolam hydrochloride 5 mg/mL diluted and 20 mL aqua pro injection to obtain a 1 mg/mL level test solution. Then, 1 mL solution was stored in a sterile vial container.

This study also revealed the effect of storage temperature on the stability of midazolam hydrochloride reconstitution preparations. Three solutions test were stored at room temperature (20-25 °C) and at refrigerator temperature (3-4 °C). At the time of testing, the sample was taken by 1 mL from the storage container using aseptic techniques. The sampling point in this study was carried out at 0 o'clock (shortly after preparation), 8, 24, and 48 hours after preparation.

Preparation Stability Test

The samples were taken at 0, 8, 12, and 24 hours after preparation, then evaluated for physical, chemical, and microbiological stability. Physical stability tests was performed, including observe color's solution and it's clarity. The solution clarity was tested by placing a sample above lighting and observed by rotating the sample and flipping the container against a black or white background (Erweka Apparatus Gmens Hensen Stamn, Germany). Thus, it could clarity and the presence of foreign particles in the test sample were observed.

The sample was taken by 5 mL for physical stability checks. Microbiological stability tests were examined by the sterility requirements of intravenous preparations. Sterility tests were conducted on bacterial growth media in microbiology laboratories. Chemical stability tests was examined including pH testing and levels determination

using HPLC. PH measurement was performed because the pH can affect the preparation stability. the pH measurements of midazolam preparations after storage was conducted by using a pH meter (Eutech Instruments pH 700, Germany) at 25 ˚C.

Midazolam Hydrochloride Analysis Method Using HPLC

Midazolam hydrochloride levels were quantitatively measured using HPLC. The analysis conditions were as follows: the stationary phase uses an HPLC C18 column, the detector used was a photodiode array (PDA), and the analysis was analyzed at a wavelength of 220 nm, the mobile phase was acetonitrile 0.3 M potassium dihydrogen phosphate pH 3.3 (3:7 v/v) and a flow rate of 1 mL/minute, and the injection sample volume was 10 µL

Standard solute was prepared by dissolving midazolam hydrochloride at a level of 1 mg/mL in methanol to be injected in the HPLC. Furthermore, it analyzed the peak area of the chromatogram. The results will be used to measure midazolam levels quantitatively by using a comparison of the chromatogram peak area of the sample with the standard 1 mg/mL level midazolam solution.

Statistic Analytic

For qualitative analysis, the results were compared to the initial solution visually. If there were differences, there was a qualitative change in stability. For quantitative analysis, the test solution resulted stable if midazolam hydrochloride levels in the test solution >90% initial levels. Statistical analysis used one-way analysis of variance followed by Tukey's post hoc test with a confidence level of 0.05 to determine the significance differences between all the results The test used SPSS version 23.0 (SPSS, Inc., Chicago, IL).

Results

Solution Clarity test

This study found that all preparations were clear and there were no deposits or foreign particles in the sample container (Table 1). During storage phase, the midazolam did not experience precipitation in both storage. However, there were substances in most vial samples such as fibers (unpublished data) observed in >50% of the test samples (±50 vials) which were not observed in the test samples with polypropylene syringes.

Table 1. The results of the clarity observation of the midazolam solution in Intensive Care Unit's inpatient ward Dr. Soetomo Teaching Hospital (n=3).

No	Sampling-Time	pH Midazolam injection			
		Spuit		Vial	
		Room temperature	Refgirator temperature	Room temperature	Refgirator temperature
1	0th hour	*	-	*	-
2	1st hour	*	*	*	*
3	8th hour	*	*	*	*
4	12th hour	*	*	*	*
5	24th hour	*	*	*	*

Note: * = there is no sedimentation

pH Test

All of samples did not experience significant pH changes, where the mean pH of the samples was around 4.3 to 4.5, at the 1st and 24th hours (Table 2). The test sample showed pH in the range of 4.4 as for the 0th hour. Thus, there was no change in the pH of the midazolam solution during storage, both room temperature and refrigerator temperature, proving that the relatively stable preparation was kept until the 24th hour.

Table 2. The results of the pH test in midazolam in the Intensive Care Unit inpatient ward of Dr. Soetomo Teaching Hospital (n=3).

No	Sampling-Time	pH Midazolam injection			
		Spuit		Vial	
		Room temperature	Refgirator temperature	Room temperature	Refgirator temperature
1	0th hour	4.41±0.05	-	4.38±0.05	-
2	1st hour	4.36±0.05	4.47±0.03	4.39±0.01	4.47±0.13
3	8th hour	4.41±0.06	4.42±0.05	4.40±0.05	4.50±0.02
4	12th hour	12th hour	4.49±0.08	4.45±0.06	4.58±0.01
5	24th hour	4.32±0.05	4.50±0.02	4.36±0.07	4.49±0.05

Microbiology Stability Test

The sterility test resulted that 6 test containers representing about 3% of the total sample and showed no microbial contamination in the vial container. In general, after incubation, the test results showed negative results, it can be declared as sterile. However, there was a sample from 3 replications showed a *Staphylococcus pseudintermedius* culture at 8 o'clock (Table 3).

Table 3. The results of the microbiological stability test of midazolam in Intensive Care Unit's inpatient ward Dr. Soetomo Teaching Hospital (n=3).

No	Sample	Storage temperature	Microbiological Test Results at the hour			
			0	1	12	24
1	Spuit	Room temperature	Negative	Negative	Negative	Negative
		Refgirator temperature	-	Negative	Negative	Negative
2	Vial	Room temperature	Negative	Negative	Negative	Negative
		Refgirator temperature	-	Negative	Negative	Negative

Stability Test for Midazolam Levels

By using a comparative analysis of the chromatogram peaks of the raw midazolam material. A quantitative level of the drug sample was obtained (Table 4).

Table 4. HPLC test results on drug levels of midazolam injection samples after 24 hours in the Intensive Care Unit's inpatient ward of Dr. Soetomo Teaching Hospital (n=3).

Sampling-Time	Midazolam Level (Persecent)			
	Spuit		Vial	
	Room temperature	Refgirator temperature	Room temperature	Refgirator temperature
24th hour	90.31±7.59	91.97±19.93	95.68±3.14	96.85±8.92

The level of midazolam at the 0 minute was lower than the data at the 24th hour, both stored at room temperature and refrigerator. After 24 hours, the levels of medicinal ingredients were still 90.31%-96.85%, both samples stored in vials and polypropylene syringes. There were no significant differences between the storage temperature in the room and the refrigerator. Moreover, midazolam concentration were in the range of 95% after 48 hours in refrigerators, either by using vial containers or injection syringes. At this point, samples at room temperature were not tested due to the technical problems. The coefficient between measurement replications was still too large (in range 3.28%-21.67%).

Discussion

This study proved that midazolam hydrochloride injection, which was prepared using aseptic technique, has good stability until the 24th hour after the manufacturing process. Both glass vial and injection syringes didn't show significant effect on drug content as well as 24-hours storage temperatures. Previous research explained that midazolam stability was influenced by pH, storage temperature, light, and storage containers[6]. This study found that the midazolam injection was still stable, both physically, chemically, and microbiologically until the 24th hour after its mixture. Recent study showed stable diluted concentration of midazolam was 0.03 mg/mL to 0.5 for 24 hours with 5% dextrose or 0.9% sodium chloride injection[10]. On the other hand, midazolam 5 mg/mL was stable at room temperature for 100 days in polypropylene syringes[11].

Treatment of samples was divided into 2 categories which used different containers, sterile glass vials and injection syringes containing polypropylene. The results showed that there was no significant difference between midazolam injection which prepared in sterile glass vial containers and injection syringes in any storage conditions. PH measurement showed unchanged results between observation times, which around 4.3-4.6 with stable physical conditions and observed the deposition of drug ingredients during storage. Moreover, more than 90% of the initial concentration of midazolam can be observed in injection samples at 24 hours after preparation, even at 48 hours in samples stored in the refrigerator. This data was in line with the previous studies which have shown that midazolam solution was stable even up to 5-weeks after manufactured and there was no midazolam adsorption process in injection syringe containers[5]. In the 0-hour sample, the results of the determination of the levels were smaller than the 24th hour which was about 81-86%. This was possible because of over filling after the dilution process of 5 mg/mL midazolam levels with aqua pro injection to a level of 1 mg/mL which results in a diluted final concentration.

In addition, sterility tests for injection samples have also been revealed. This is an important parameter for preparations of parenteral routes. The results showed that most of the samples showed sterility to germs/microbes. However, there were 1 in 3 sample replications which showed contamination by *Staphylococcus pseudintermedius* contained in the free air. This was possible because there was of working technique on a sample that less accurate due to drugs preparation.

Thus, it was necessary to limit the number of midazolam injection samples to avoid incorrect technique, so that will obtain perfect sterility. Besides, there were several contaminations, such as fine fibers in most of the glass vial container samples which were not found in the injection syringe container. This was the main evaluation in the sterile vial sterilization process.

These data were expected to be a guide or reference for the preparation process of midazolam injection in hospitals, especially Dr. Soetomo Teaching Hospital, and generally other hospitals in Indonesia with proper aseptic techniques. Further studies needs to concern about dose uniformity after the dilution process as important information for proper preparation of midazolam hydrochloride in the ward. This was an attempt to ensure dose uniformity uniformity between injection sample, which this study found that the coefficient of variation between replications was still too large, around 3-22%.

Conclusion

This study showed midazolam hydrocloride injection had good physical, chemical, and microbiological stability until the 24th hour after the manufacturing process. Vial and injection syringes did not show a significant effect on drug content in 24-hours storage temperature. However, midazolam injection was possible to be contaminated by microorganism because of less accurate technique during preparation.

Acknowledgement

This research was funded by the Surabaya City Ministry of Health Fund for the 2017 financial year. Thank you to the Dr. Seotomo Teaching Hospital who has facilitated the implementation of this research, especially in the Pharmacy-Hospital Installation, Dr. Microbiology Department, and *BALITBANG*, Surabaya. Furthermore, gratitude was also said to Universitas Airlangga, Faculty of Pharmacy's Testing Services Unit (ULP) for assisting in the determination of the midazolam level with HPLC, as well as all parties who helped to conduct this research well.

Ethical Clearance: This study has been approved ethically by the Medical Research Ethics Committee of Faculty of Medicine, Universitas Airlangga Surabaya.

Conflict of Interest: There is no conflict of interest

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