Original Article

Long-Term Stability of Lorazepam in Sodium Chloride 0.9% Stored at Different Temperatures in Different Containers

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M. L. Colsoul[1], A. Breuer[1], N. Goderniaux[1], J. D. Hecq[1], L. Soumoy[1], B. Bihin[1], J. Jamart[1], and L. Galanti[1]

Abstract

Background and Objective: Infusion containing lorazepam is used by geriatric department to limit anxiety disorders in the elderly. Currently, these infusions are prepared according to demand by the nursing staff, but the preparation in advance in a centralized service could improve quality of preparation and time management. The aim of this study was to investigate the long-term stability of this infusion in polypropylene syringes stored at 5 ± 3°C. Then, results obtained were compared with stability data of lorazepam in syringes stored at room temperature, glass bottles at 5 ± 3°C, and glass bottles at room temperature. **Method:** Eight syringes and 6 bottles of infusion were prepared by diluting 1 mL lorazepam 4 mg in 23 mL of NaCl 0.9% under aseptic conditions. Five syringes and 3 bottles were stored at 5 ± 3°C and 3 syringes and 3 bottles were stored at room temperature for 30 days. During the storage period, particle appearance or color change were periodically checked by visual and microscope inspection. Turbidity was assessed by measurements of optical density (OD) at 3 wavelengths (350 nm, 410 nm, 550 nm). The stability of pH was also evaluated. The lorazepam concentrations were measured at each time point by high-performance liquid chromatography with ultraviolet detector at 220 nm. **Results:** Solutions were physically unstable in syringes at 5 ± 3°C after 4 days: crystals and a drop of OD at 350 nm were observed. However, pH was stable. After 2 days, solutions were considered as chemically unstable because a loss of lorazepam concentration higher than 10% was noticed: the lower 1-sided confidence limit at 95% was below 90% of the initial concentration. To assess temperature and polypropylene influence, results were compared with those obtained for syringes at room temperature and bottles at 5 ± 3°C and room temperature. Precipitation, drop of OD at 350 nm, and chemical instability were observed in all conditions. **Conclusion:** Solutions of lorazepam were unstable after 2 days in syringes at 5 ± 3°C. Preparation in advance appears, therefore, not possible for the clinical use. Storage conditions (temperature and form) do not improve the stability.

Keywords
admixture programs/incompatibilities, drug stability, medication therapy management (MTM), psychotherapeutics

Introduction

Lorazepam, a part of the benzodiazepine family, is commonly recommended in the elderly to relieve anxiety and to produce sedation.[1,2] Benzodiazepines modulate positively the opening of the gamma amino butyric acid (GABA)-A receptor, a chloride-selective ion channel whose natural ligand is GABA. The GABA bonding results in a decrease of excitability of neurons and thus produces a calming effect on the brain.[3]

Lorazepam is mainly used in geriatric unit and is gradually administered to the patient by a syringe driver. Currently, the preparation of syringes at therapeutic concentration is carried out according to demand by the geriatric staff. To improve time management and preparations quality, these infusions could be prepared by a Centralized Intravenous Additive Service.[4,5] However, this goal cannot be achieved without knowing the infusion stability. Some data are available about the lorazepam stability in glass bottles,[6,7] in polyvinyl chloride bags,[2,8] in polyolefin bags,[2,9] and in polypropylene syringes.[7,10] These data show considerable variations of lorazepam stability depending on container and storage temperature. Nevertheless, the long-term stability of lorazepam diluted in NaCl 0.9% at therapeutic concentration and stored in syringes at 5 ± 3°C remains unknown. The aim

[1]CHU UCL Namur, Yvoir, Belgium

Corresponding Author:
J. D. Hecq, Drug Stability Research Group, CHU UCL Namur, 1 avenue Therasse, Yvoir 5530, Belgium.
Email: jean-daniel.hecq@uclouvain.be

of this study is to determine the physicochemical stability of lorazepam 167 μg/mL in polypropylene syringes at 5 ± 3°C and to compare results with stability data of lorazepam in syringes stored at room temperature, in glass bottles at 5 ± 3°C, and in glass bottles at room temperature.

Method

Solutions Preparation

Eight polypropylene syringes (Becton Dickinson, lot 1709211, Madrid, Spain) and 6 glass bottles were prepared under aseptic conditions. Each was prepared by diluting 1 mL of lorazepam solution 4 mg/mL (Temesta®; Pfizer, lot 4070, Brussels, Belgium) in 23 mL of sodium chloride solution (NaCl 0.9%; Baxter, lot 18A14G60, Lessines, Belgium) to obtain a final lorazepam concentration of 167 μg/mL.

Storage

Five syringes and 3 bottles were stored at 5 ± 3°C. Three syringes and 3 bottles were stored at room temperature. Immediately after the preparation (day 0) and after 1, 2, 3, 4, and 7 days, 1 mL of each solution was withdrawn from each syringe and bottle to perform the physical stability tests while 20 μL intended for chemical stability evaluation were frozen at 80°C.

Physical Stability

At each time point, samples were visually inspected with the unaided eye in front of a black and a white background to check the absence of particle, haze, precipitation, and color change. The samples were also centrifuged at 2150 g for 5 minutes (Heraeus multifuge 1S; Thermo Scientific, Waltham, Massachusetts) before observing the pellet under an optical microscope 12.5× (Jenamed; Carl Zeiss, Germany) to search crystals.

The optical densities (ODs) were measured with a spectrophotometer (Genesys 10 UV; Spectronic Unicam, Thermo Scientific, Waltham, Massachusetts) at 350, 410, and 550 nm to detect subvisible particles and assess turbidity of solution.[11]

The pH of each sample was measured with a glass electrode pH meter (InoLab; WTW GmbH, Germany).

Chemical Stability

Chromatographic conditions

A high-performance liquid chromatography (HPLC) system connected to an ultraviolet (UV) detector (Alliance 2695, detector 2489; Waters, Milford, Connecticut) and a processing software (Empower 3 software; Waters) was used.

The chromatographic separation was performed on a reverse phase column (Synergi 4μm Hydro-RP 80A 150 × 4.6 mm; Phenomenex, Torrance, California) with a mobile phase containing 65% of KH_2PO_4 (Merck, lot A0360673202, Darmstadt, Germany) 10 mM pH 2.4 (adjusted with phosphoric acid 85 wt. % in water, Sigma-Aldrich, lot MKCB7708, St. Louis, Missouri) and 35% of acetonitrile (Biosolve, lot 1228451, Dieuze, France). The flow rate was set at 2.5 mL/min, the column temperature at 30°C, and the detection wavelength at 220 nm.

Five standard solutions (26, 50, 99, 160, 200 μg/mL of lorazepam) and 3 control levels (26, 99, 200 μg/mL) were prepared before each analysis. Fifty microliters of 1/10 diluted calibrators, controls, and therapeutic solutions were analyzed during a run time of 7 minutes. The elution time of lorazepam was 4 minutes.

Validation of the HPLC Method

The method was validated according to precision, linearity, limit of detection (LOD), limit of quantification (LOQ), and stability indication, as recommended in the ICH Q2(R1) guidelines.[12]

Precision. The within- and between-day variations were calculated based on 3 levels of lorazepam concentration (approximately 26, 99, and 200 μg/mL) with 10 repetitions for each level.

Linearity. Linearity was evaluated by the analysis of 14 solutions obtained from the 2-fold serial dilution in water of a 200 μg/mL lorazepam solution.

LOD and LOQ. Ten blank (mobile phase) measurements were used to determine LOD and LOQ. They were calculated as follows:

$$\text{LOD} = \text{mean} + 3\text{SD}.$$

$$\text{LOQ} = \text{mean} + 10\text{SD}.$$

Stability Indication. The stability-indicating capability of the chromatographic method was assessed using partially decomposed solutions of lorazepam. The solution was analyzed in natural (pH 5.8), acidic (adjusted at pH 1.7 with HCl 5M), and basic (adjusted at pH 12.3 with NaOH 5M) conditions before and after heating at 100°C for 1 hour.

Chemical Stability Study

All frozen samples withdrawn from a same container were injected the same day in HPLC to reduce results variability due to experiment conditions.

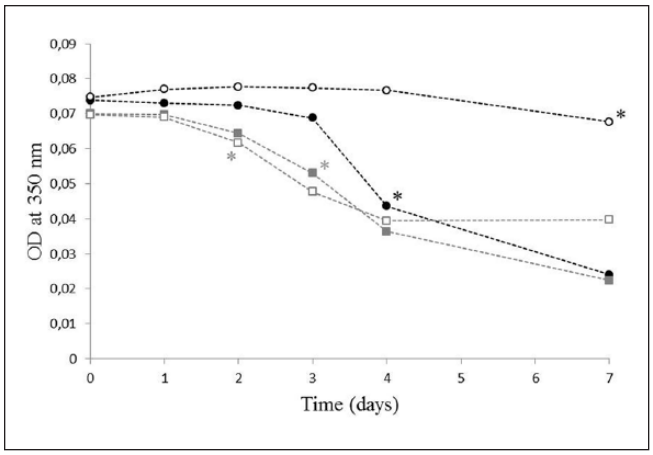

Figure 1. Mean of optical densities at 350 nm versus time in syringes at 5 ± 3°C (filled black circle), in syringes at room temperature (empty black circle), in glass bottles at 5 ± 3°C (filled gray square), and in glass bottles at room temperature (empty gray square).
Note. Asterisks show the days when crystallization occurred. OD = optical density.

Statistical Analysis

Solutions were considered chemically stable if the one-sided 95% confidence interval on the mean concentration remains higher than 90% of the initial lorazepam concentration[13] or 95% of the initial lorazepam concentration if any signs of physical instability occurred.[14]

Results

Physical Stability

After 4 days, crystals were detected with the unaided eye and microscope in syringes stored at 5 ± 3°C. A decrease of absorbance measured at 350 nm was observed with crystallization (Figure 1). Another storage condition (at room temperature) and another container (glass bottle) were tested but crystals appeared with a decreasing of OD at 350 nm (mean ± SD: 0.060 ± 0.019) in each condition: after 7 days in syringes at room temperature, 3 days in bottles at 5 ± 3°C, and 2 days in bottles at room temperature (Figure 1). For all syringes and bottles included in the study, the measurement of pH at each time did not show any change during the storage (6.6 ± 0.1). There was no change of color and absorbance at 410 nm (0.002 ± 0.002) and 550 nm (0.001 ± 0.001).

Validation of the HPLC Method

The analytical response was linear between 0.05 and 200 µg/mL of lorazepam with a determination coefficient of 0.999. The relative SD representing inter- and intra-assay precision was lower than 3.04% (Table 1). Forced degradation test proved the stability-indicating capability of the method given that degradation products were observed without interference with lorazepam peak (Figure 2). The LOD and LOQ were determined by calculation and were 0.004 µg/mL and 0.011 µg/mL, respectively.

Table 1. Precision for Intra- and Inter-Assay for Lorazepam (n = 10).

	Intra-assay			Inter-assay		
	Control 1	Control 2	Control 3	Control 1	Control 2	Control 3
Mean, µg/mL	24.133	95.041	196.787	24.988	98.739	198.913
SD, µg/mL	0.075	0.575	1.776	0.759	1.422	3.198
Relative SD, %	0.311	0.605	0.903	3.039	1.440	1.607

Chemical Stability

The lower limit of the one-sided 95% confidence interval on the mean concentration remained higher than 90% of the initial lorazepam concentration for 2 days for syringes stored at 5 ± 3°C (Figure 3a) and for 4 days for the ones stored at room temperature (Figure 3b). This limit was higher than 90% for only one day for solutions in glass bottles stored at 5 ± 3°C and at room temperature (Figure 3c and 3d).

Discussion

Results showed that lorazepam solutions in polypropylene syringes stored at 5 ± 3°C were physically stable for only 3 days given that crystals appeared on the fourth day. Initial absorbance of solutions at 350 nm was higher than the ones at 410 nm and 550 nm suggesting that a component present in the therapeutic solutions, such as lorazepam, absorbs at this wavelength. Interestingly, a drop of OD at 350 nm was observed while crystallization occurred, indicating that this component could be precipitated. To confirm that absorbance at 350 nm was due to lorazepam and that the precipitates observed were lorazepam crystals, crystals were redissolved in mobile phase before HPLC analysis. Chromatogram displayed a peak corresponding to lorazepam at about 4 minutes. Precipitation of lorazepam can be explained by the limited solubility of this drug in aqueous solution; this is why the commercial solution contained an organic solvent (polyethylene glycol, propylene glycol, and benzyl alcohol).[15,16]

The influences of temperature and polypropylene on crystallization were assessed by evaluating the physical stability of lorazepam solutions in syringes at room temperature and in bottles at 5 ± 3°C and at room temperature. There is no influence of temperature or propylene on crystallization given that precipitation occurred in all cases. Furthermore, previous stability studies in other container, in other diluents, and at other temperature also related lorazepam crystallization.[6,7,8,10,15]

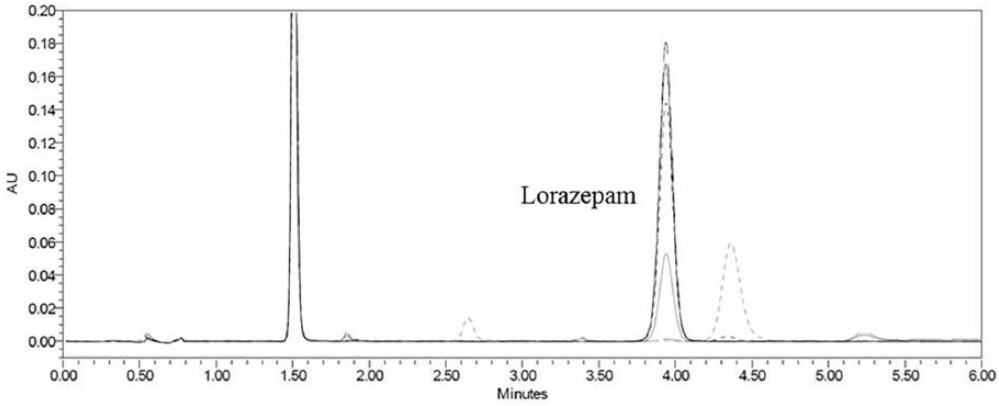

Figure 2. Forced degradation chromatograms of lorazepam before (black) and after heating (gray) at natural (solid line), acidic (dashed line) and basic (dotted line) pH.

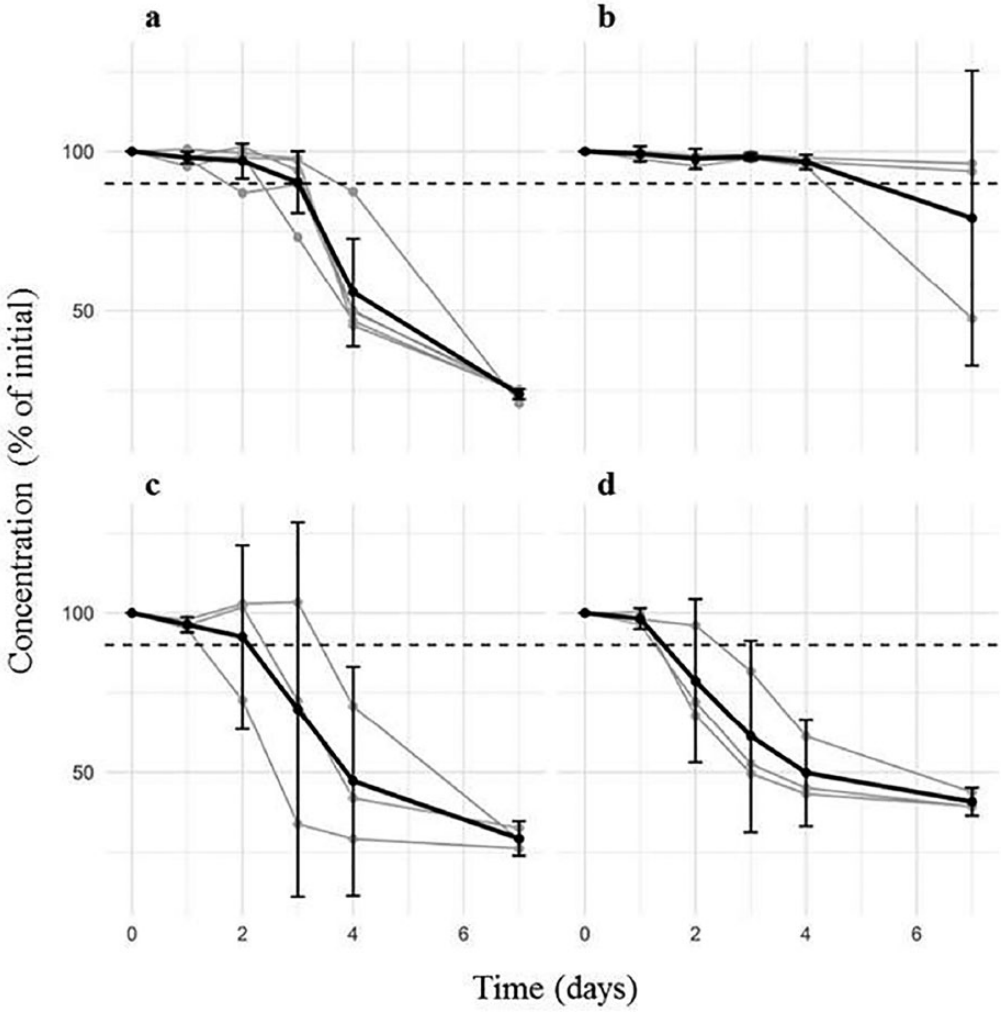

Figure 3. (a) Relative concentration of lorazepam versus time in syringes at 5 ± 3°C, (b) syringes at room temperature, (c) bottles at 5 ± 3°C, and (d) bottles at room temperature.

Note. gray dots = observations for each studied solution; black dots = observations means; dotted line = 90% of the initial concentration.

Chemical stability results showed that lorazepam solutions were stable for 2 days in syringes at 5 ± 3°C and for 4 days in syringes at room temperature while solutions in bottles at both 5 ± 3°C and room temperature were stable for only 1 day. No sterility test was performed.

Conclusion

Physicochemical stability of lorazepam 167 µg/mL in polypropylene syringes stored at 5 ± 3°C was evaluated to consider the preparation of syringes in advance by a centralized service. This could contribute to the global management of treatments by providing geriatric unit with ready-to-use injectable drugs for relieving patients' anxiety.

This study showed that the physicochemical stability of lorazepam is limited at 2 days in propylene syringes. Indeed, lorazepam was not stable due to crystal appearance and a rapid decrease of lorazepam concentration.

Other conditions (temperature and container) were tested to improve stability but crystallization occurred in all cases. Therefore, lorazepam solution at 167 µg/mL in NaCl 0.9% cannot be prepared in advance, which does not allow a centralized preparation.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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