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Stability Studies of Clonazepam 2.5 mg/mL Oral Solution and 1 mg/mL Parenteral Solution in Pre-Filled Syringes

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Abstract

Background: Clonazepam is a benzodiazepine drug indicated in all clinical forms of epileptic seizures, various forms of myoclonic seizures, myoclonus and other abnormal movements. At present, it is classified as a hazardous drug for workers, according to a technical document produced by the Spanish National Institute for Safety and Health at Work (INSST), in collaboration with the Spanish Society of Hospital Pharmacy (SEFH). **Objectives**: Administration of clonazepam in pre-filled syringes connected to a closed safety system, made in the pharmacy service, may facilitate its administration and reduce the risks to the health or safety of nursing personnel. Therefore, a physicochemical stability study of clonazepam in ready-to-use pre-filled syringes for oral and parenteral administration was carried out. **Methods**: A rapid, linear, precise and sensitive high-performance liquid chromatography (HPLC) method for chemical stability studies of Clonazepam 1 mg/mL (parenteral use) and 2.5 mg/mL (oral use) in solution was implemented after repackaging in pre-filled syringes. The studies were conducted by measuring concentrations of oral and parenteral Clonazepam in pre-filled syringes, at various time points, over 30 days in several different storage conditions: oral clonazepam protected from light in refrigerator and at controlled room temperature exposed to ambient light; parenteral clonazepam protected from light in refrigerator and at controlled room temperature protected or unprotected from light. Visual aspects and pH change as well as crystal formation were checked to determine physical stability. **Results**: The loss of the active ingredient in all groups was less than 10% after 30 days. No evidence of crystal formation, pH and visual aspect changes were observed. **Conclusions**: Clonazepam 1 mg/mL parenteral solution and 2.5 mg/mL oral solution in pre-filled syringes are stable for up to 30 days in the tested conditions. The centralized repackaging of clonazepam in pre-filled syringes, connected to a closed safety system, in the pharmacy service, reduces drug manipulation by nursing staff decreasing the risk of occupational exposure.

Keywords: clonazepam; stability study; pre-filled syringes; validation; HPLC

1. Introduction

Clonazepam is a drug that belongs to the group of benzodiazepines. Its mechanism of action involves allosteric interactions between central benzodiazepine receptors and gamma-aminobutyric acid (GABA) receptors in the brain, enhancing the effects of GABA. In Spain it is indicated in most of the clinical forms of epileptic disease and seizures in infants, children and adults. In the last group it is used in status epilepticus too [1,2].

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The INSST and the American Institute for Occupational Safety and Health of the United States (NIOSH) classify it as a group 3 non-antineoplastic drug that primarily has adverse reproductive effects [3,4]. The FDA classified clonazepam as a category "D" pregnancy risk drug prior to 2015 [5].

A pre-filled syringe is a ready-to-use system that decreases the hazards of drug manipulation and also saves nursing time. Nevertheless, currently the lack of stability studies of clonazepam in pre-filled syringes prevents the pharmacy services from preparing and storing it. Therefore, this study investigates the physicochemical stability of clonazepam in pre-filled syringes in several different conditions.

2. Materials and Methods

2.1. Sample Preparation of Pre-Filled Syringes

Oral clonazepam 2.5 mg/mL solution syringes: Amber polypropylene 1 ml light protected oral syringes (Becton DickinsonTM, Madrid –Spain-) with a tip cap were pre-filled with 0.4 mL of clonazepam 2.5 mg/mL oral solution drops (Rivotril®, Roche Farma, S.A., Madrid, -Spain-). Each syringe contained 1 mg of clonazepam (Figure 1). Two groups of syringes were stored at either controlled room temperature (25ºC) exposed to ambient light, or under refrigerated conditions (2-8ºC) protected from light.

Figure 1. BD™ Oral syringe loaded with 0.4 mL of clonazepam 2.5 mg/mL.

Parenteral clonazepam 1 mg/mL solution syringes: Luer lock polypropylene syringes (Nipro Europe Group Companies, Madrid –Spain-) for parental use were pre-filled with 1 mL of clonazepam 1 mg/mL parenteral solution (Rivotril® powder 1 mg + 1 mL solvent, Roche Farma, S.A., Madrid, -Spain-), connected to a closed safety system (TexiumTM, Becton Dickinson España, S.A., Madrid, -Spain-) as shown in figure 2. Two groups of syringes were stored at either controlled room temperature (25ºC) protected or unprotected from light, or under refrigerated conditions (2-8ºC) protected from light.

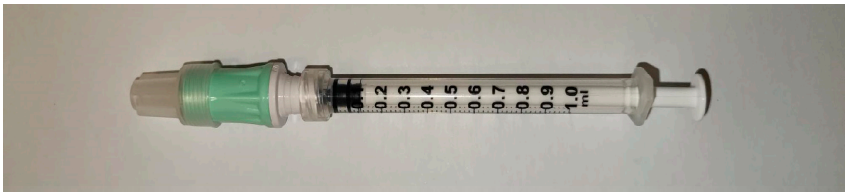

Figure 2. Nipro luer-lock syringe with closed safety system to load with 1 mL of clonazepam 1 mg/mL.

2.2. Chemical Stability

The chemical stability of oral and parenteral clonazepam in pre-filled syringes was studied over 30 days of storage (day 0; days 1 to 4; days: 7, 9, 11, 14, 17, 21, 24, 28 and 30).

The chemical stability was studied on the selected days by withdrawing an aliquot of each syringe that was then diluted with the mobile phase to a concentration of 25 µg/mL. Three different batches of each preparation were analyzed in triplicate by HPLC within 10 minutes of dilution.

If the drug concentration remained between 90 and 110% of the initial concentration during the 30 days of storage, the preparation was considered stable [6–8].

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2.3. Chromatographic Method

Conditions: A Waters Breeze HPLC system (Waters Cromatography, S.A., Barcelona, -Spain-) and a XBridge 5 µm C18 (130 Å pore size, 4.6 x 150 mm) reversed-phase column (Waters Cromatography, S.A., Barcelona, -Spain-) were used. The chromatographic conditions were: isocratic mobile phase composed of ultrapure water/acetonitrile/methanol (40/30/30 v/v) at a flow rate of 1 mL/min, ultraviolet detector at 254 nm, 30°C column temperature, injection volume of 20 µL and run time of 5 minutes [9,10]. The HPLC reagents acetonitrile (HPLC-grade) and methanol (HPLC-grade) were purchased from Panreac Química S.L.U. (Barcelona, -Spain-). The reference drug clonazepam was obtained from Roche Farma, S.A. (Madrid, -Spain-).

Validation of the method: The HPLC method was validated in terms of linearity, precision and accuracy according to the ICH guidelines [11]. **Linearity:** Linearity between the peak area and the clonazepam concentration was evaluated by performing five measurements in a concentration range of 6-45 µg/mL (6, 15, 24, 30 and 45 µg/mL). A calibration curve and the corresponding linear regression analysis were performed, obtaining the results of the coefficient of determination (R^2), the slope (a) and the Y intercept (b) [6]. **Precision:** Precision was verified by repeatability in intra and inter-day studies. The intra-day study consisted of analyzing five times, on the same day, the samples at 80, 100 and 120% of the target concentration (25 µg/mL). In the inter-day study the samples were analyzed five times during four different days, at 80, 100 and 120% of the target concentration. The mean, the standard deviation and the coefficient of variation were calculated, with less than 1% variation being accepted for intra-day repeatability, and less than 2% for inter-day repeatability [6,12]. **Accuracy:** Accuracy was determined by recovery studies in triplicate at 20, 25 and 30 µg/mL concentrations of clonazepam. Recovery was expressed as a percentage, and the mean value was compared with the theoretical value (100%), using Student's t test [6, 12,13]. **Limit of detection and limit of quantification:** To determine the limit of detection (LOD) and the limit of quantification (LOQ) of clonazepam, the independent term "b" and the slope "a" were used in the equations LOD = 3 b/a and LOQ = 10 b/a [6,14].

2.4. Physical Stability

Physical stability was studied checking visual aspects, determining pH and observing crystal formation. 1 mL samples of each preparation were obtained on the selected evaluation days and were checked for visual aspects such as particle formation, crystals, turbidity, precipitation or color changes during storage. The pH was measured with a calibrated SevenMultiTM pH meter (Mettler Toledo, Cornellà de Llobregat, -Spain-). A visual inspection booth with both a black/white background [15] and bright-field, and a SediMAX2TM phase contrast microscope (77 Elektronika, Budapest, -Hungary-), were used to determine the presence of particles and crystals.

3. Results

3.1. Validation of the Analytical Method

The method demonstrated excellent linearity, with a R^2 greater than 0.9996. The regression equation was calculated as y = 127436x + 8625. The results were highly satisfactory regarding the intra-day and inter-day repeatability of the three clonazepam quality control solutions. Accuracy ranged from 98.34% to 101.62%, while precision, expressed as relative standard deviation (RSD%), fell within the range of 0.094% to 0.682% for intra-day precision and 0.232% to 0.713% for inter-day precision. These RSD% values comfortably meet the ICH (International Conference on Harmonisation) standards, which stipulate a maximum RSD of 1% and 2% respectively. Likewise, the accuracy was between 98% and 102%. The LOD and the LOQ were calculated as 0.20 µg/mL and 0.68 µ/mL respectively (Table 1-2).

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Table 1. Intra-day repeatability of clonazepam samples at 80, 100 and 120% of the target concentration of 25 µg/mL.

Theoretical Concentration (µg/mL)	20 (80%)	25 (100%)	30 (120%)
Mean (µg/mL)	20,04	24,99	30,06
SD	0,03	0,02	0,21
RSD%	0,15	0,09	0,68
Accuracy%	100,20	99,98	100,12

SD= Standard deviation; RSD%: Relative standard deviation.

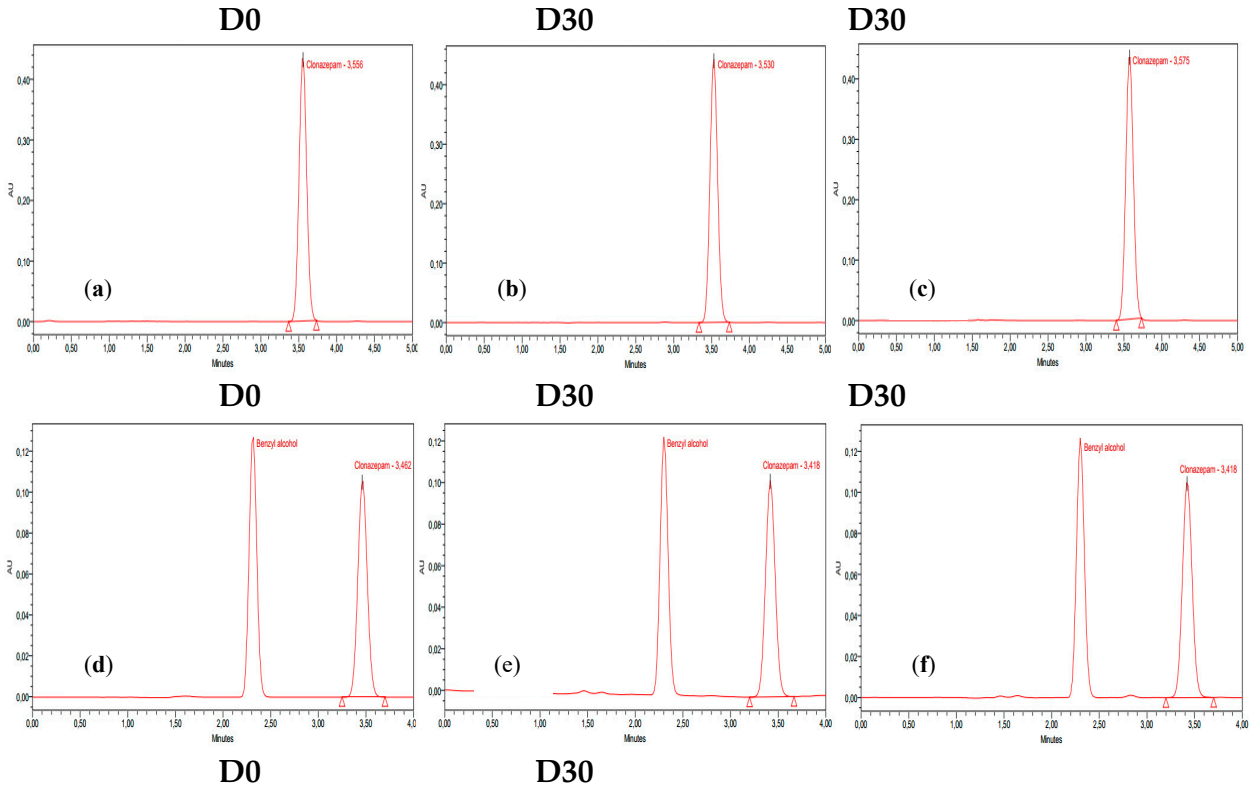
Table 2. Inter-day repeatability of clonazepam samples at 80, 100 and 120% of the target concentration of 25 µg/mL.

Theoretical Concentration (µg/mL)	20 (80%)	25 (100%)	30 (120%)
Mean (µg/mL)	20,04	25,04	30,10
SD	0,07	0,06	0,21
RSD%	0,33	0,23	0,71
Accuracy%	100,21	100,15	100,32

SD= Standard deviation; RSD%: Relative standard deviation.

3.2. Stability Study

The stability study was carried out by measuring the concentration of clonazepam in pre-filled syringes on each day of the analysis, as described previously. The mean concentrations were calculated and expressed as recovery percentage with respect to the measurement on the first day (D0 = 100%). The chromatograms of D0 and D30 (day 30 of the study) for each preparation under the specified storage conditions are presented in Figure 1. The clonazepam 1 mg/mL parenteral solution contains benzyl alcohol as a preservative agent. This compound exhibits absorption at 254 nm, which accounts for the appearance of a corresponding peak in the chromatograms.



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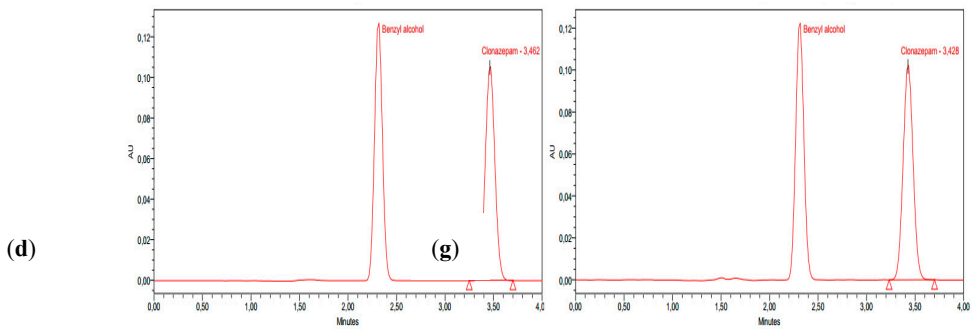

Figure 1. Chromatograms of clonazepam. Oral clonazepam 2.5 mg/mL solution in pre-filled syringes: (**a**) D0; (**b**) D30 room temperature; (**c**) refrigeration condition protected from de light. Parenteral clonazepam 1 mg/mL solution in pre-filled syringes: (**d**) D0; (**e**) room temperature; (**f**) room temperature protected from light; (**g**) refrigeration condition protected from de light.

The results show that the concentrations remained stable for 30 days in all of the storage conditions used for the oral clonazepam 2.5 mg/mL solution syringes (Table 3) and the parenteral clonazepam 1 mg/mL solution syringes (Table 4).

No significant variation was observed with regards to visual aspects (color changes, turbidity) and pH throughout the study (Table 3 and Table 4).

Table 3. Physicochemical results of oral clonazepam 2.5 mg/mL solution in pre-filled syringes.

	Room temperature		**Refrigeration conditions Protected from light**	
	D0	**D30**	**D0**	**D30**
Average Recovery % of concentration	100	100,33±0,01	100	97,82±0,02
pH	4,63±0,02	4,65±0,06	4,65±0,03	4,65±0,05
Colour	Blue	Blue	Blue	Blue
Crystals ≥10 µm/mL	0	0	0	0

Results expressed as mean ± SD (standard deviation) of triplicate determinations.

Table 4. Physicochemical results of parenteral clonazepam 1 mg/mL solution in pre-filled syringes.

	Room temperature	Room temperature protected from light	Refrigeration conditions protected from light
	D0	**D30**	**D30**	**D30**
Average Recovery % of concentration	100	100,87±0,01	98,14±0,02	98,02±0,02
pH	4,15±0,08	4,27±0,15	4,30±0,07	4,17±0,04
Colour	Transparent	Transparent	Transparent	Transparent
Crystals ≥10 µm/mL	0	0	0	0

Results expressed as mean ± SD (standard deviation) of triplicate determinations.

4. Discussion

Since the publication of the document on hazardous drugs and preventive measures for their preparation and administration, Hospital Pharmacy Services have been involved in implementing measures to adapt practices to the recommendations given by the INSST and European Agency for Safety and Health at Work in its guidance document for the safe management of hazardous medicinal

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products at work [16]. Among the adaptation options is the possibility of direct delivery of the drug from the Pharmacy Service in a standardized dose and container, ready for administration. If stability studies of the drug in standardized containers are available, it is possible to prepare and store the drug in the Pharmacy Service, depending on consumption, to avoid the need for shift or daily preparation.

There are currently only two studies published that evaluate the stability of oral clonazepam solutions. One of them assessed the stability of clonazepam 0.2 mg/mL oral solution stored under refrigeration (2-8ºC) and at room temperature, using clonazepam in powder form, concluding that the solution was stable for 90 days. The other analyzed the stability of clonazepam 0.1 mg/mL oral solution prepared from commercial tablets, both stored under refrigeration (2-8ºC) and at room temperature protected from light, observing that the solution remained stable for 60 days under both storage conditions. Unlike the previous cases, this study has evaluated the stability of commercialized clonazepam drugs in the presentations of 2.5 mg/mL oral drops and 1 mg/mL injectable solution, repackaged in pre-filled polypropylene syringes. Both concentrations are significantly higher than the concentrations mentioned in the studies beforehand, a condition that does not seem to affect the stability observed in the current work.

5. Conclusions

In the present study, clonazepam 2.5 mg/mL oral solution in light-protected pre-filled polypropylene syringes, both at room temperature and under refrigeration (2-8ºC), and clonazepam 1 mg/mL parenteral solution in pre-filled polypropylene syringes at room temperature with and without light protection, and under refrigeration (2-8ºC) with light protection, are observed to be physically and chemically stable for at least 30 days. This has allowed for preparation of ready-to-use stock of this hazardous drug, minimizing drug manipulation by nursing staff and therefore reducing the risk of occupational exposure.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

GABA	Gamma-aminobutyric acid
HPLC	High-performance liquid chromatography
ICH	International Conference on Harmonisation
INSST	Spanish National Institute for Safety and Health at Work
LOD	Limit of detection
LOQ	Limit of quantification
NIOSH	Health of the United States
R2	Correlation coefficient

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RSD%	Relative standard deviation
SD	Standard deviation
SEFH	Spanish Society of Hospital Pharmacy

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