Stability of Diazepam Enema Extemporaneous Formulation in "Manzoni" Base

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Abstract

We report the preparation of an extemporaneous liquid formulation of diazepam enema for both paediatric and adults patients using an alcohol-free base solution. Chemical stability of the drug in this formulation has been performed by using a high-pressure liquid chromatography analytical method. Results have showed that diazepam was chemically stable when the formulation was stored at 25 °C over a period of 3 months.

Introduction

Diazepam is a drug of the benzodiazepine family that has anticonvulsant, sedative, and muscle relaxant properties (Figure 1). It is used in the treatment of severe anxiety and tension states, as a sedative and premedication, in the control of muscle spasm, and in the management of alcohol withdrawal symptoms. This active pharmaceutical ingredient (API) may be used for acute severe anxiety and agitation, epileptic and febrile convulsions, tetanus, as a sedative in minor surgical and dental procedures, or in other circumstances in which a rapid effect is required but where intravenous injection is impracticable or undesirable.[1]

INSERT FIGURE 1

This drug can be formulated as an enema and dedicated for emergency situations such as convulsions in infants, children and adults.[2]

In the last period (from mid-2020), the commercial product in Italy Micropam® (Aurobindo Pharma s.r.l.) has become unavailable (as evidenced by various patient reports and requests from the Italian pharmacists), leaving patients (children and adults) who need it without that treatment. Galenic pharmacists can overcome this problem by being able to produce extemporaneous formulations, including diazepam micro-enemas, in their pharmacy laboratories.[3,4] Hence the need to evaluate the possibility of preparing an alternative extemporaneous liquid formulation, ease to prepare and administer to paediatric and adults patients.

In this study we have described the preparation of an extemporaneous liquid formulation of diazepam enema for paediatric patients using an alcohol-free commercial base solution, "Manzoni". Chemical stability of the API in that formulation has been performed by using a high-pressure liquid chromatography (HPLC) as analytical method. Results have showed that the API was chemically stable when the formulations were stored at 25 °C for 3 months.

Materials and Methods

Diazepam and "Manzoni" base solution (composition: propylene glycol, PEG 400, preserved water, hydroxyethyl cellulose) were obtained from Farmalabor srl (Canosa di Puglia, Italy). All other chemicals used were analytical-grade quality from Merck (Milan, Italy).

Preparation of enema containing diazepam

Extemporaneous solutions of diazepam were prepared by dissolving the drug in "Manzoni" base solution as described. Briefly, the volume of the vehicle needed to set up the drug solution was measured with a graduated cylinder and transferred in a glass backer. The drug was exactly weighed, added to the base and dissolved under constant magnetic stirring at 40 °C for 1 hour. The resulting formulations were prepared three times, aliquoted in 8 mL glass vials and stored at 25 °C in a climatic chamber (Climacell, MMM Medcenter-Munico Germany) until analysis. The vehicle volume was set up to obtain a solution containing diazepam at a final concentration of 1.67 mg/mL (5 mg/3 mL, 10 mg/6 mL).

HPLC method and stability studies

The liquid chromatograph consisted of an Agilent 1260 Infinity quaternary LC VL system equipped with a variable wavelength detector and with openLab DCS software. A Hyperclone C_{18} ODS 5 µm 120 Å 250 x 4.6 mm column thermostated at 35 °C was used. An isocratic separation was performed using as mobile phase water acidified with H_2PO_4 at pH 3 (25%) and methanol (75%) at a flow rate of 1.1 mL/min. UV detection was carried out at 254 nm. An injection volume of 20 mL was used in all experiments. The amount of diazepam in the collected samples was measured by the chromatograph peak area in relation to those obtained from the standards samples analysed under the same conditions. 0.5 mL aliquots of the solution, after shacking of the formulation, from each of the stored samples at time zero and after 2 weeks, 1, 2, and 3 months, were collected, centrifuged at 13.200 rpm for 10 min at 25 °C and filtered (nylon filter, pore size 0.45 µm). 0.1 mL of the filtered solution of each sample was diluted with mobile phase into a 10 mL volumetric flask and vigorously mixed for 10 seconds using a vortex before being analysed by HPLC.

Standard curves and linearity

1 mg/mL stock solution of the API was prepared using the mobile phase. This solution was used to prepare serial dilutions. The linearity of the method was demonstrated using five different drug concentrations in a range between 5 and 100 µg/mL. Each concentration peak area was recorded in triplicate, and taken average area from triplicate injections. The linearity was determined by least squares regression analysis. The calibration line was described by the equation y = 35002x + 2.9. The coefficient of correlation was 0.9989. The retention time of the peak was 5.0 min and the analysis time for one run was 10 min. The lower limit of detection (LOD) and lower limit of quantification (LOQ) for diazepam were calculated based on the standard deviation of the response of the curve (Sy) and the slope of the calibration curve at levels approximating the LOD according to Eq 1 and LOQ to Eq 2:

$$\mathrm{LOD} = 3.3\frac{\mathrm{Sy}}{\mathrm{Slope}} \qquad (1)$$

$$\mathrm{LOQ} = 10\frac{\mathrm{Sy}}{\mathrm{Slope}} \qquad (2)$$

The LOD and LOQ was 0.98 μg/mL and 2.98 μg/mL respectively.

Results

The preparation of an extemporaneous liquid formulation of diazepam enema for paediatric patients using an alcohol-free base solution named "Manzoni" has been described. The new liquid base allowed us to dissolve the API at a final concentration of 1.67 mg/mL. Chemical stability of diazepam in that formulation has been evaluated by using a HPLC analytical method. Table 1 reports the data on drug assay obtained storing extemporaneous liquid formulations of diazepam at 25 °C. No changes of colour and pH values (~9.0) was observed for these formulations during storages at 25 °C for 3 months.

INSERT TABLE 1

Discussions

A new alcohol-free base solution named "Manzoni" has been used to prepare an extemporaneous formulation of the antiepileptic drug diazepam. HPLC analysis has showed that the API dissolved in the vehicle at a concentration of 1.67 mg/mL was chemically stable when the formulation was stored at 25 °C for 3 months (Table 1). This concentration allows the extemporaneous preparation of diazepam enema for both paediatric (5 mg/3 mL) and adults (10 mg/6 mL) patients. The proposed diazepam-based extemporaneous liquid formulation administer as enema has been easily prepared and could represent an alternative formulation for the management of seizures in paediatric patients when intravenous access cannot be obtained or commercial products are unavailable.

Conclusion

The preparation of an extemporaneous liquid formulation of diazepam enema for both paediatric patients and adults using an alcohol-free base solution has been described. HPLC analysis has confirmed the chemical stability of the API when the formulation was stored at 25 °C for 3 months. This extemporaneous formulation could be easily prepared in a galenic pharmacy and represent a valuable and safe alternative to commercial products.

References

1. Calcaterra NE, Barrow JC. Classics in chemical neuroscience: diazepam (valium). *ACS Chem. Neurosci*. 2014; 5(4): 253-60.

2. Jannin V, Lemagnen G, Gueroult P, et al. Rectal route in the 21st Century to treat children. *Adv. Drug Deliv. Rev.* 2014; 73: 34-49.

3. Perrone M, Laquintana V, Lopedota AA, et al. Stability data of extemporaneous suspensions of hydroxychloroquine sulphate in oral liquid bases after tablet manipulation, *Data Brief* 2000; vol. 33, 106575.

4. Lopalco A, Curci A, Lopedota A, et al. Pharmaceutical preformulation studies and paediatric oral formulations of sodium dichloroacetate. *Eur J Pharm Sci*. 2019; 127: 339-350.

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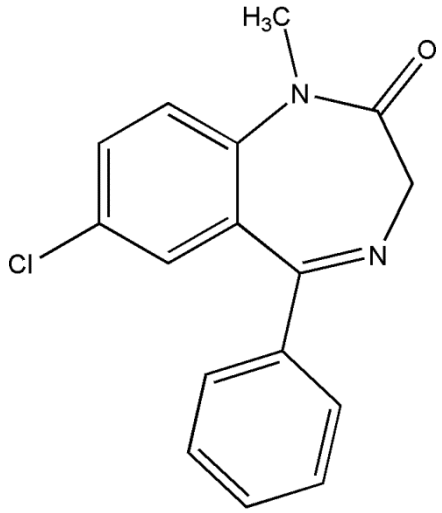

Figure 1. Molecular structure of Diazepam.

Table 1. Data on the chemical stability of diazepam solubilized in “Manzoni” base solution when stored at 25°C for 3 months. Data are expressed as mean of tree analysis and standard deviation (SD).

Sampling times (months)	Diazepam drug assay (mg/mL)	SD (mg/mL)
0	1.68	0.11
0.5	1.65	0.13
1	1.60	0.17
2	1.69	0.12
3	1.63	0.10