Stability of Cefazolin Sodium Eye Drops

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ABSTRACT

In this study, the influence of storage temperature on the stability of cefazolin sodium in Tears Naturale II (33 mg/ml) was evaluated. Cefazolin sodium was reconstituted in Tears naturale II and the solutions stored at 4°C and 28°C for 28 days. The effects of different storage temperatures on the stability and microbial contamination were examined.

The fortified stock solutions of cefazolin sodium were prepared by reconstituting with water for injection and with Tears Naturale II. The stability was evaluated by measuring the absorbance spectrum and pH. During the study period, the levels of contamination of all of the solutions were examined by tryptic soy broth for 24-48 hours.

Throughout the 28-day period, no change was observed in the percentage of the labeled amount of cefazolin sodium stored at 4°C, but the percentage at 28°C decreased after 7 days ($p<0.05$). The pH of the eye drops was in the range of 3.5-10.5 which is usually tolerable by the eyes. No contamination was found in any of the solutions during the study period.

The main conclusions to be drawn from this study are that if topical fortified cefazolin sodium solutions are to be used for longer than 7 days, they should be stored at 4°C while those stored at 28°C should be discarded after 7 days.

Key words: Cefazolin sodium eye drops

INTRODUCTION

Depending on the seriousness of the condition, bacterial eye infections (e.g., corneal ulcers, keratitis) need "strengthened eye drops" containing a high concentration of antibiotics (Steinert, 1991). Cefazolin sodium eye drops (33 mg/ml) are administered topically to the eye to treat microbial keratitis (Tananuvat et al., 2004). As these are not commercially available, they are made up and prescribed for the treatment of eye infections due to sensitive bacterial combinations after isolation. The Division of Pharmacy, Chiang Mai University, routinely makes up eye drop preparations, containing 33 mg/ml cefazolin sodium. It has been reported that parenteral solutions of cefazolin sodium, following reconstitution with water for injection, should be discarded after 24 hours storage at 25°C or after 96 hours at

5°C (Bornstein et al., 1974). Stability studies of other pharmaceutical solutions of cefazolin sodium in various artificial tear solutions and aqueous vehicles have shown that these solutions should be used within 3 days if stored at room temperature. In addition, it is advisable to store the reconstituted products in a refrigerator during use (Ahmed and Day, 1987).

Cefazolin sodium (33 mg/ml) eye drops have to be prepared extemporaneously from cefazolin sodium injections under aseptic conditions. Due to the lack of stability data, eye drops are prepared upon receipt of an order. Therefore, the aims of this study were to study stability, pH and microbial safety of cefazolin sodium 33 mg/ml eye drops when stored at room temperature (28°C) and at 4°C.

MATERIALS AND METHODS

Chemicals and reagents

Cefazolin sodium for injection and Tear Naturalle II were purchased from Fujisawa Pharmaceutical, Japan and Alcon Laboratories, respectively. A pure reference standard of cefazolin sodium was purchased from Sigma Chemical Company, USA.

Equipment

The instruments used to measure pH and the absorbance spectra were a pH meter (Orion Research Incorporated) and a UV spectrophotometer (Shimadzu), respectively.

Preparation of standard solutions

A standard stock solution of cefazolin sodium (0.33 mg/ml) was prepared. A further 6 solutions were prepared by dilution of 150, 250, 450, 650, 750 and 800 ul of the cefazolin sodium (0.33 mg/ml) stock solution with distilled water and the volumes adjusted to 10 ml. Thus, solution concentrations of 4.95, 8.25, 14.85, 21.45, 24.75 and 26.40 ug/ml were obtained. Each solution was then analysed by the UV spectrophotometer to set up a standard curve.

Preparation of eye drops for stability studies

Twenty solutions of cefazolin sodium for injection were each reconstituted with water for injection and with artificial tear vehicle (Tear Naturalle II) to a concentration of 33 mg/ml in Tear Naturalle II containers. The containers were wrapped in aluminum foil and divided into two groups. Ten bottles were stored in the dark at room temperature (28°C) while the other ten bottles were kept at 4°C in a refrigerator.

On day 0, 3, 7, 10, 14, 21 and 28, the absorbance spectrum and pH of each solution were measured. The pH measurements were carried out immediately after removing the samples from storage. All solutions were diluted with distilled water to give an absorbance reading of less than 1.00. The absorbance was measured from the prominent peak at 272 nm for cefazolin sodium (Arici et al., 1999).

Preparation of eye drops for microbiological contamination studies

Fourteen solutions of cefazolin sodium for injection were each reconstituted aseptically with water for injection and with artificial tear vehicle (Tear Naturalle II) to a concentration of 33 mg/ml under laminar air flow in Tear Naturalle II containers. The containers were wrapped in aluminum foil and divided into two groups. Seven bottles were stored in the dark at room temperature (28°C) while the other seven were kept at 4°C in a refrigerator.

On day 0, 3, 7, 10, 14, 21 and 28, 1 ml samples were taken from one of the room- temperature containers and one of the refrigerated containers and added into a tryptic soy broth to enrich the microbial growth, if present, by an aseptic technique. Further samples were inoculated onto blood agar and Sabouraud agar to detect bacterial and fungal contamination, respectively. The bottles of tryptic soy broth and the blood agar plates were incubated at 37°C while the Sabouraud agar plates were kept at 30°C. After overnight incubation, 0.5 ml aliquots from the inoculated tryptic soy broth were sub-cultured onto blood agar plates and anaerobic blood agar plates and incubated at 37°C to detect contamination. All of the plates were carefully examined after 7 days for microbial growth.

Statistical analysis

Measurements are given as mean ± standard deviation (SD). Statistical analysis of the data was performed using SPSS 11.0 for Windows One-Way Anova and Dunnett Test. Different significant percentages of the labeled amounts between day 0 and day 3, 7, 10, 14, 21 and 28 at room temperature and 4°C were determined. Results with $p<0.05$ were considered to be statistically significant.

Table 1. Percentage of the labeled amounts of cefazolin sodium 33 mg/ml eye drops.

Storage time (days)	Percentage of the labeled amountsa	
	4°C	28°C
0	104.81 ± 2.09	103.22 ± 1.41
3	102.20 ± 2.80	99.46 ± 2.47
7	101.62 ± 2.87	98.92 ± 2.88
10	101.74 ± 2.11	92.70 ± 2.22*
14	103.51 ± 4.21	92.42 ± 7.39*
21	104.78 ± 2.84	91.44 ± 4.33*
28	104.80 ± 4.01	83.80 ± 4.07*

[a]mean ± SD of 10 samples

*P< 0.05

Table 2. Mean pH and contamination.

		Storage time (days)						
		Day 0	**Day 3**	**Day 7**	**Day 10**	**Day 14**	**Day 21**	**Day 28**
4°C	Mean pH	6.44	6.41	6.23	6.34	6.30	6.25	6.20
	Contamination	No growth	No growth	No growth	No growth	No growth	No growth	No growth
28°C	Mean pH	6.46	6.22	6.29	6.56	6.61	6.59	6.68
	Contamination	No growth	No growth	No growth	No growth	No growth	No growth	No growth

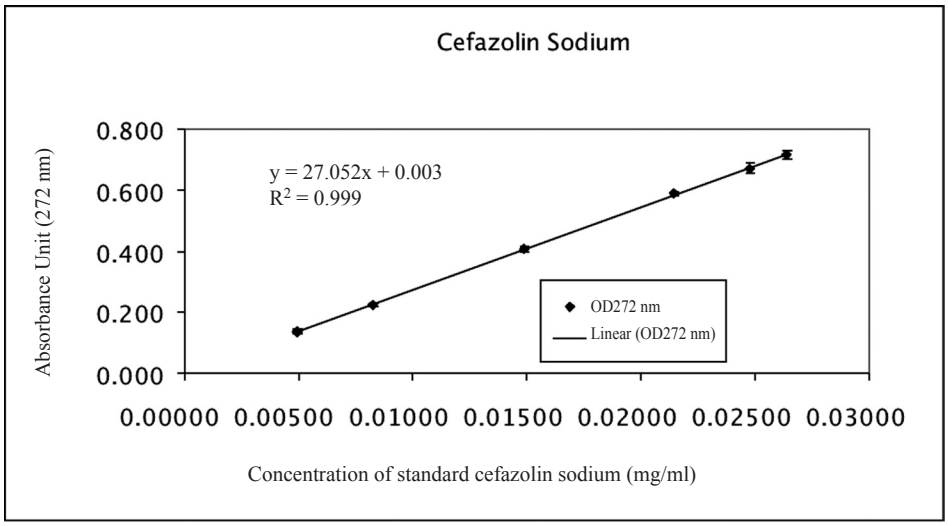

Figure 1. Standard calibration curve.

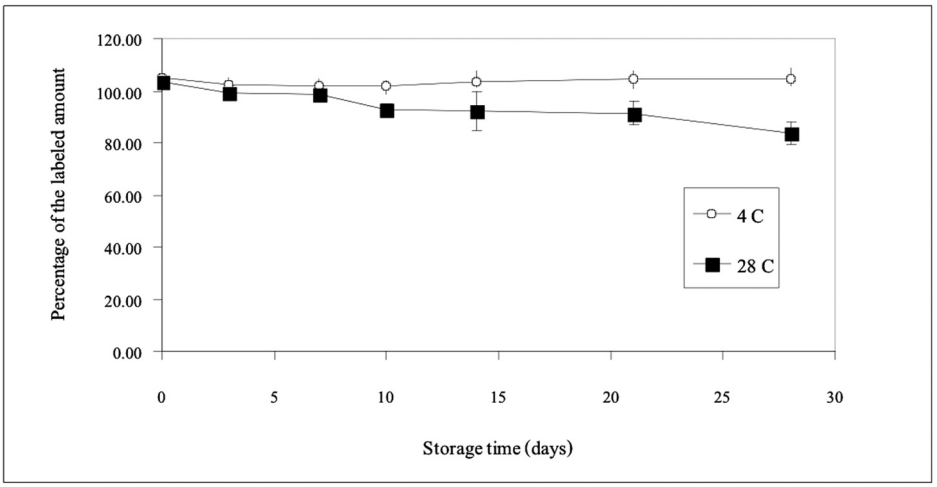

Figure 2. Percentage of the labeled amounts at 4°C and 28°C.

RESULTS AND DISCUSSION

Linearity of the cefazolin sodium calibration curve, ranging from 4.95 to 26.4 ug/ml, was seen from correlation coefficients of more than 0.999 in all assays. The standard calibration equation is

$$y = 27.052x + 0.003 \text{ (Figure 1)},$$

in which y is absorbance units and x is the concentration of the cefazolin sodium solution. The concentration of the cefazolin eye drops was calculated using this equation.

The percentage of the labeled amount was calculated as

$$100\ C1 / C2$$

where C1 is the concentration of cefazolin sodium at day 0-28 in mg/ml and C2 is the concentration of the standard cefazolin sodium.

The percentage of the labeled amount of cefazolin sodium in the eye drops stored at 4°C indicated no loss of stability during 28 days. However, at 28°C, there was a statistically significant decrease in the percentage of the labeled amount from day 10 onwards ($p<0.05$) (Table 1, Figure 2). The pH of the eye drops was in the range of 3.5-10.5 (Table 2) which is usually tolerable by the eyes (Lund, 1994). No growth of aerobes, anaerobes or fungi in all of the eye drops, both at 4°C and 28°C, was observed after 48 hours of incubation (Table 2). This indicates that there was no contamination present in the eye drops prepared under the aseptic conditions of the hospital pharmacy.

CONCLUSIONS

The results of this study demonstrate the stability of 'strengthened' ophthalmic solutions of cefazolin sodium eye drops (33 mg/ml) for 28 days at 4°C and 28°C. The stability was maintained when the solutions were stored for 28 days in the refrigerator at 4°C. However, there was a loss of stability from day 10 onwards when the solutions were stored at 28°C. Consequently, it is recommended that cefazolin 33 mg/ml eye drops should be discarded after 7- day storage at room temperature or after 28 days under refrigeration. This recommendation is made to reduce the potency and potential for the growth of microorganisms and to minimize the increase in color and change in pH of cefazolin sodium eye drops.

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