7. Procedures

Both eyes of each animal were examined on the start date of the test to ensure the absence of ocular abnormalities.

Before 5 minutes of instillation, 1 to 2 drops of 0.4 % oxybuprocaine hydrochloride were instilled in both eyes for local anesthesia. Next, 0.1 mL of the test sample was instilled into the conjunctival sac of one eye of each animal. The lids were then gently held together for about 1 second. The other eye, which was not treated with the test sample, was used as a control.

The cornea, iris, and conjunctivae were observed clinically using a hand slit-lamp (× 10, Ohira Co., Ltd.) at 1, 24, 48 and 72 hours after the instillation. The ocular reactions were observed and scored according to the criteria of Draize method shown in Table 1. The corneal epithelium was examined further in detail with the use of fluorescein sodium at each observation time except for 1 hour after the instillation.

The total score was calculated for each animal according to the formula shown in Table 2, and the mean of the total scores of all the animals was obtained at each observation time. The eye irritation potential of the test sample was graded by means of the maximum mean total score on the basis of Table 3.

The animals were weighed before treatment and on the last observation date.

8. Results (Tables 4 to 8)

1) Rabbit No. 1

In the test eye, redness of both palpebral and bulbar conjunctivae (both score 1) was seen at 1 hour after instillation. At the 24-hour observation, the reaction had disappeared. No irritant response was observed during the subsequent period.

On the other hand, the control eye showed no abnormalities throughout the observation period.

No fluorescein staining was detected in either the test eye or the control eye throughout the observation period.

2) Rabbit No. 2

The test eye or the control eye showed no abnormalities throughout the observation period.

No fluorescein staining was detected in either the test eye or the control eye throughout the observation period.

3) Rabbit No. 3

The test eye or the control eye showed no abnormalities throughout the observation period.

No fluorescein staining was detected in either the test eye or the control eye throughout the observation period.

The maximum mean total score was 0.7 (at 1 hour after instillation) in the test eyes and 0 in the control eyes.