Stability of Vancomycin 10, 25 and 50 mg/mL Ophthalmic Drops in Tears Naturale II Stored in Low Density Polyethylene Dropper Bottles at 4 and 25°C for 30 days

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INTRODUCTION

Vancomycin ophthalmic drops are the treatment of choice for ocular infections caused by methicillin-resistant *Staphylococcus aureus.* There is no stability data for vancomycin ophthalmic solutions when prepared in Tears Naturale II.

OBJECTIVES

The objective of the study was to evaluate the stability of vancomycin 10, 25, and 50 mg/mL reconstituted with Tears Naturale II over 30 days at 4°C and 25°C in low density polyethylene (LDPE) eye dropper bottles.

METHODS

Liquid Chromatographic Method

The liquid chromatographic system consisted of 7% acetonitrile and 93% 0.05 M phosphoric acid which was pumped through 150 mm x 4.6 mm reverse-phase, 5 µm column (Agilent Zobax SB-CN, Toronto, ON) at 1.0 mL/min. The effluent was monitored with UV detection (Waters 998 photodiode array detector, Toronto, ON) at 280 nm.

Assay Validation

The method was evaluated to ensure reproducibility, accuracy and specificity. The system was shown to be capable of separating vancomycin from its degradation products. The accuracy and reproducibility of standard curves was tested over 5 days. Inter- and intra-day errors of reproducibility were assessed by the coefficients of variation (CV) and the standard deviation of regression.

Stability Study

On study day 0, 24 vials of vancomycin 1 g lyophilized powder (Sterimax, lot: 37615Y, Exp: 08/2024) were reconstituted with 20 mL of Tears Naturale II (Alcon, lot: 154H6F, exp: 2025/09) to yield a 50 mg/mL solution. The 25 mg/mL and 10mg/mL solutions were prepared by drawing up the reconstituted solution and further diluting with Tears Naturale II. Six syringes of each concentration were filtered with a 5 µm sterile filter into 30 mL LDPE dropper bottles (MPS Pharma+, lot: M6285, exp: 2027/03/14) and two syringes of each concentration were filtered with a 5 µm sterile filter into a glass vial (ALK, lot: SEV2101122, exp: 01/2026). Three LDPE dropper bottles and one glass vial of each concentration were stored at 4°C and 25°C.

Vancomycin concentrations from each dropper bottle were measured on study days 0, 1, 4, 8, 14, 21, 24, and 30. The concentration of vancomycin was measured using a validated, stability indicating liquid chromatographic method using UV detection. The glass vials were inspected for physical changes on each study day against a white and black background.

Data Reduction and Statistical Analysis

The concentration of a solution on a particular day was considered "acceptable" or "within acceptable limits" if it was greater than 90% of the initial concentration (as determined on day 0) with 95% confidence. Chemical stability was calculated using the lower limit of the observed degradation rate with 95% confidence and the time to achieve 90% of the initial concentration.

Analysis of variance was used to test differences in degradation rate between storage temperature, initial concentration and study day. The 5% level was used as the *a priori* cut-off for significance.

RESULTS

Table 1. Percent Remaining of Initial Vancomycin Concentration[1] on Each Study Day.

Temperature		25°C	25°C	25°C	4°C	4°C	4°C
Nominal Concentration (mg/mL)		50	25	10	50	25	10
Actual Concentration (mg/mL)		54.17	25.91	10.06	54.64	25.93	10.54
Study Day	0	100	100	100	100	100	100
	1	100.72 ± 0.83	99.91±1.16	102.57 ± 3.36	98.65 ± 0.04	99.20±0.53	98.44 ± 0.55
	4	96.97 ± 0.64	98.36±1.18	101.90 ± 3.59	97.92 ± 0.85	100.44±0.30	97.81 ± 0.27
	8	96.41 ± 0.39	97.87±1.24	102.73 ± 3.27	99.55 ± 0.49	102.29±1.01	99.08 ± 1.08
	14	94.87 ± 0.76	95.52±0.94	99.85 ± 3.06	97.69 ± 0.83	103.61±1.72	99.48 ± 0.90
	21	94.20 ± 0.78	93.73±1.12	98.28 ± 3.25	99.30 ± 0.32	98.82±0.32	101.69 ± 0.38
	24	92.28 ± 0.69	92.76±1.21	97.27 ± 3.09	99.78 ± 1.08	98.87±0.35	101.09 ± 1.64
	30	90.85 ± 1.09	91.46±1.32	96.11 ± 3.64	97.75 ± 0.41	98.04±0.16	101.42 ± 1.04
Rate of Concentration Change (%/day)		-0.295	-0.292	-0.192	-0.015	-0.064	0.103
Intercept		99.554	99.920	102.292	99.027	100.973	98.567
Correlation		-0.964	-0.996	-0.877	-0.183	-0.379	0.814
Standard Deviation of Regression (sy.x)		0.997	0.299	1.287	1.011	1.906	0.895
Std Error in Slope (Sb)		0.033371	0.009996	0.043046	0.033810	0.063781	0.029939
Confidence Interval for slope		0.08166	0.02446	0.10533	0.08273	0.15607	0.07326
Fastest Slope 95% Confidence		-0.3770	-0.3161	-0.2978	-0.0982	-0.2200	0.0296
Upper Limit95% Confidence		-0.2137	-0.2672	-0.0871	0.0673	0.0921	0.1761
Shortest T-90 (95% CI)		26.52	31.63	33.58	101.86	45.46	-338.23

1. Concentrations are shown as mean ± coefficient of variation (CV), expressed as a percentage

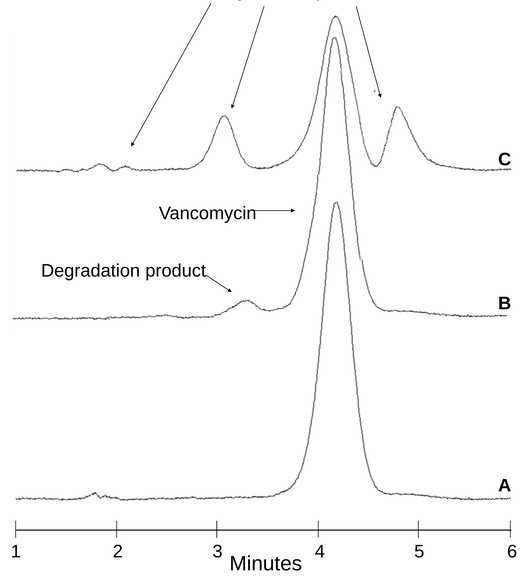
Assay Validation

Assay validation demonstrated that degradation products are separated from vancomycin (Figure 1). The assay was accurate as measurement of the standards and quality control samples averaged an absolute deviation of 1.59% from the expected concentration. Within day replicate error averaged 0.44% and between day replicate error averaged 1.33%. A second measure of replicate error, the standard deviation of regression, averaged 1.07.

Figure 1. Representative Chromatograms

Chromatogram A represents a 50 mg/mL on study day 0. Chromatogram B was observed after 30 days of storage at 25°C. 90.85% of the initial concentration remained.

Chromatogram C represents a solution of vancomycin after degradation with heat (87°C) for 7 hours with 54.05% remaining. Degradation products were noted to elute at 1.9, 2.2, 3.2 and 4.9 minutes.



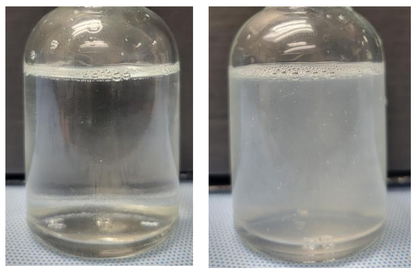


Figure 2. Physical inspection of vancomycin 50 mg/mL in Tears Naturale II in glass vial against black background.

Vancomycin 50 mg/mL in Tears Naturale II stored at 4°C on study day 0 (left) and study day 30 (right). On study day 21, the vial had a slightly cloudy appearance, which then continued to progress until study completion.

Concentration Results

The initial concentration and percent remaining on each study day are listed in Table 1. The calculated time to achieve 90% of the initial concentration exceeded the 30 day study period for all concentrations and temperatures except the 50 mg/mL solution, where the calculated time was 26 days.

Analysis of variance identified significant differences in percent remaining due to study day (p=0.03), temperature (p=0.001) and initial concentration (p=0.01).

Physical Inspection

The 10 and 25 mg/mL ophthalmic drops remained clear and colourless at 4°C and 25°C for the entire study duration. The 50 mg/mL ophthalmic drops were clear and colourless when stored at room temperature. However, the samples stored at 4°C began to develop a cloudy appearance on day 21 which persisted for the remainder of the study (Figure 2).

CONCLUSIONS

Vancomycin 10, 25, and 50 mg/mL ophthalmic drops in Tears Naturale II are chemically stable for 30 days when stored at 4°C and 26 days when stored at 25°C.

The 50 mg/mL ophthalmic drops were physically stable for only 14 days when stored in the fridge.

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Karen Lam – Nothing to disclose
Romina Marchesano – Nothing to disclose
Shirley Law – Nothing to disclose
Alba Caku – Nothing to disclose
William Perks – Consultant for Medisca Pharmaceutique Inc.
Nathan H Ma – Nothing to disclose

This study was funded by the Department of Pharmacy, Sunnybrook Health Sciences Centre