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Impact of deep freezing on the stability of 25 mg/ml vancomycin ophthalmic solutions

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Abstract

For the treatment of certain eye infections, ophthalmic solutions 'laced' with 25 mg/ml vancomycin are sometimes prepared. Their physical and chemical stability and the maintenance of their sterility were studied after deep freezing at -20 ± 2 °C and thawing, followed or not by refrigeration for 48 h at 4 ± 2 °C. Physical and chemical analysis comprised visual inspection turbidity, determination of pH and osmolality, and assay of vancomycin by high performance liquid chromatography with ultraviolet detection. For microbiological analysis a 25 mg/ml vancomycin ophthalmic solution was filtered through two membranes and cultured on trypticase-soy and Sabouraud-glucose solid media. Any colonies were then counted. These physical, chemical and microbiological analyses demonstrated the stability of 25 mg/ml vancomycin ophthalmic solutions in 5% glucose deep frozen at -20 ± 2 °C for 3 months. The vancomycin concentration varied by no more than 5% of the initial concentration, and no breakdown product was evidenced. Neither pH (mean = 3.8 ± 0.1) nor osmolality (mean = 318.3 ± 5.6 mOsm/kg) varied significantly, and remained compatible with intraocular administration. No particle or bacterial combination was found in the course of the study. The thawing procedure (at ambient temperature or under warm running water from a tap) did not modify the stability of the eye drops. Likewise, storage in a refrigerator for 48 h after thawing did not modify stability. The advantage of storing vancomycin 25 mg/ml ophthalmic solutions for 3 months in deep freeze is that a stock of chemically and microbiologically controlled preparations can be held ready for administration to patients, thereby allowing prompter dispensing, as the eye drops are not made up extemporaneously, while the improved control over production ensures that patients receive solutions of constant quality, as every batch prepared is systematically inspected.

Keywords: Vancomycin; Ophthalmic solutions; Stability; Deep freezing

1. Introduction

Depending on the seriousness of the condition and the bacterial combinations implicated, certain

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eye infections (e.g. corneal ulcers, keratitis) need 'strengthened eyedrops' containing a high concentration of antibiotics (Steinert, 1991; Sauvageon-Martre et al., 1993). As these are not commercially available, they are made up ad hoc, and prescribed for the treatment of eye infections due to sensitive bacterial combinations after isolation and antibiogram recording. The Clermont–Ferrand Hospital Pharmacy is often called upon to make up preparations containing 25 mg/ml vancomycin. Vancomycin is a glycopeptide that offers the advantage of a fairly high intracorneal penetration, a wide spectrum covering Gram-positive bacteria (including methicillin-resistant staphylococci) and no cross-resistance with other antibiotics (Fiscella, 1995).

As the prescription of such 25 mg/ml vancomycin ophthalmic solutions is increasingly common, the Control and Development Laboratory of the Clermont–Ferrand Hospital Pharmacy studied their stability after deep freezing to determine whether they could be prepared in advance so as to be immediately available for emergency eye treatment.

Some authors have evaluated the stability of ophthalmic solutions containing high concentrations of vancomycin at ambient temperature, after refrigeration, and/or after deep freezing (Charlton et al., 1998; Clifton Fuhrman and Stroman, 1998; Arici et al., 1999; Barbault et al., 1999; Peyron et al., 1999). However, we have not found in the literature any work carried out in conditions of clinical use, in particular when thawing is performed rapidly (under warm running water and not at ambient temperature). Accordingly, we undertook to evaluate the impact on this preparation of deep freezing followed by thawing (slow at ambient temperature and fast in warm water at about 38 °C) and then refrigeration for 48 h. To this end we monitored the physical and chemical stability of the drug solution and the maintenance of its sterility.

2. Materials and méthods

2.1. Preparation of ophthalmic solutions

The ophthalmic solutions were prepared under a laminar air flow hood. To make up 34 ophthalmic solutions we needed:

- Four 500-mg bottles of freeze-dried vancomycin (Dakota Pharm®).
- Four 20-ml vials of injectable 5% isotonic glucose (Lavoisier).
- One 30-ml syringe (Omnifix, Braun) fitted with a needle (Microlance 3, Becton–Dickinson).
- Four Millex filters of porosity 0.22 pm (Millipore).
- One 100-ml sterile flask.
- Thirty-four 5-ml coloured glass bottles sterilised beforehand in a steam autoclave (20 min at 120 °C).

The 25 mg/ml vancomycin solution was obtained by dissolving the contents of each bottle of 500 mg of vancomycin in 20 ml of 5% glucose. The four solutions of 20 ml were combined in the sterile flask, thoroughly homogenised by shaking, and distributed equally, after sterilising filtration through 0.22 μm filters, in the 34 5-ml bottles, which were then closed with rubber stoppers and crimped aluminium seals. The 5% glucose solution was chosen for dilution solvent because it affords a 25 mg/ml vancomycin ophthalmic solution with a pH and osmolality that are compatible with intraocular administration. The airtightness of the containers was checked visually by turning over the stoppered sealed bottles.

2.2. Stability study design

The stability study was conducted on three series of 17 pairs of 25 mg/ml vancomycin ophthalmic solutions prepared at intervals of 3–4 days. For each pair of ophthalmic solutions one bottle was used for physical and chemical analysis, and the other for microbiological testing (in what follows concerning thawing and analysis the term 'ophthalmic solution' will be employed for simplicity, even though pairs of ophthalmic solutions were actually used).

Each series underwent the following procedure: immediately after preparation (D0) one of the 17 ophthalmic solutions was analysed (the results obtained served as reference). A second ophthalmic

solution was placed in a refrigerator and analysed on D2, i.e. after 48 h refrigeration. The other 15 ophthalmic solutions were deep frozen at -20 ± 2 °C. After 7, 14, 30 and 90 days storage they were taken out of the refrigerator in groups of three. For each group of three ophthalmic solutions, one was analysed immediately after rapid thawing under warm running water at about 38 °C, the second was analysed after thawing at ambient temperature, and the third was placed in a refrigerator at 4 ± 2 °C for 48 h before analysis.

Table 1
Design of the stability study for 25 mg/ml vancomycin ophthalmic solutions

Days	Actions
D0	Preparation of ophthalmic solutions no. 1–17 Analysis of ophthalmic solution no. 1 Deep freezing of ophthalmic solutions no. 3–17 Refrigeration of ophthalmic solution no. 2
D2	Analysis of ophthalmic solution no. 2
D7	Rapid thawing and analysis of ophthalmic solution no. 3 Thawing at ambient temperature and analysis of ophthalmic solution no. 4 Refrigeration of ophthalmic solution no. 5
D9	Analysis of ophthalmic solution no. 5
D14	Rapid thawing and analysis of ophthalmic solution no. 6 Thawing at ambient temperature and analysis of ophthalmic solution no. 7 Refrigeration of ophthalmic solution no. 8
D16	Analysis of ophthalmic solution no. 8
D30	Rapid thawing and analysis of ophthalmic solution no. 9 Thawing at ambient temperature and analysis of ophthalmic solution no. 10 Refrigeration of ophthalmic solution no. 11
D32	Analysis of ophthalmic solution no. 11
D60	Rapid thawing and analysis of ophthalmic solution no. 12 Thawing at ambient temperature and analysis of ophthalmic solution no. 13 Refrigeration of ophthalmic solution no. 14
D62	Analysis of ophthalmic solution no. 14
D90	Rapid thawing and analysis of ophthalmic solution no. 15 Thawing at ambient temperature and analysis of ophthalmic solution no. 16 Refrigeration of ophthalmic solution no. 17
D92	Analysis of ophthalmic solution no. 17

The study plan is outlined in Table 1. The stability time of 48 h in a refrigerator after thawing was set to allow for an imperative in clinical use of strengthened ophthalmic solutions: it is important to be able to use solutions thawed on Friday throughout the following week-end. For an ophthalmic solution to be safe it must be sterile, isotonic to tears, and free of injurious particles (i.e. limpid) and irritants, and have a pH that is compatible with the eye. The analysis of each ophthalmic solution therefore comprised:

- A microbiological analysis to check sterility.
- A physical and chemical analysis including evaluation of the turbidity of the solution, determination of its osmolality and pH, and assay of vancomycin.

The design of these different tests is set out in Table 1.

2.3. Microbiological analysis

This involved filtering the ophthalmic solution through a membrane and then seeding the membrane on a solid culture medium. The filtration was carried out using single-use kits (Nalgene analytical test filter funnel, 0.45 μm, 100 ml, Brand Products). A 1-ml aliquot of ophthalmic solution was filtered on the kit membrane, which was rinsed three times with 50 ml of sterile water to inhibit the action of the antibiotic and allow any microbial growth to occur. This operation was repeated once. One of the membranes was seeded on trypticase-soy agar and incubated at 30–35 °C for 7 days: this culture medium evidences bacteria. The other membrane was placed on Sabouraud-glucose agar (Laboratoires Biomérieux) for 7 days at ambient temperature: this medium favours the growth of fungi and molds. The choice of culture media, temperatures and incubation times was based on the Pharmacopée Européenne, 1997 3rd ed. Any bacterial

combinations were then counted under binocular magnification, and identified. For the trypticase-soy medium a first recording was made after 48 h incubation, and a second one after 7 days. As the agar tended to dry after 7 days at 30–35 °C we took an intermediate reading.

2.4. Physical and chemical analysis

The ophthalmic solutions were inspected visually against a white background, and on a lit white background using a tester (LR 28 PW Allen and Co.). This inspection, prescribed in the European Pharmacopoeia, evaluates the turbidity of ophthalmic solutions and the absence of suspended particles.

The pH of the solutions was measured using pH indicator reagent strips (pH range 2.5–4.5, Merck).

Osmolality was measured using a model 3MO plus micro-osmometer (Advanced Instrument) from a sample of 20 μl of ophthalmic solution.

Assay of vancomycin was carried out by high performance liquid chromatography. The analysis was performed using the following apparatus:

- Constant flow rate pump (Jasco-PU980),
- Automatic injector (Jasco-AS950-10),
- Diode array detector (L4500, Merck).
- The analysis column was a Lichrospher RP-18 column, 125×4 mm ID, 5 μm (Macherey–Nagel).

The mobile phase was a 25/60/15 (vol/vol) mixture of methanol, water and pH 3 0.01 M phosphate buffer. The pump flow rate was set at 1.2 ml/min, and the analysis wavelength at 220 nm.

The standard solutions were prepared by dilution of a parent solution of 1 mg/ml vancomycin in the mobile phase to obtain concentrations of 50, 100, 200 and 400 μg/ml. The analysis method used was validated to carry out a stability study according to the recommendations of L.A. Trissel for the stability of injectable solutions (Trissel, 1983, 1988). It specifically analyses and identifies breakdown products. For this purpose we conducted a deliberate breakdown of vancomycin by changing the pH of the solution with 5 N sodium hydroxide and then heating it strongly (boiling for 15 min). The solution obtained was then analysed by chromatography to make sure there was no interference between vancomycin and the breakdown products so generated.

The mean concentrations of vancomycin obtained at the different sampling times were calculated from the three series of ophthalmic solutions. The results are expressed as percentages relative to the initial concentration obtained on D0 (immediately after the preparation, the reference value, taken as 100%). The stability of the drug was considered satisfactory if its concentration did not diminish by more than 10% of its initial concentration (Trissel, 1998).

The effects of deep freezing, refrigeration after deep freezing and thawing procedure on stability of vancomycin were evaluated by analysis of variance (ANOVA) with a significance limit of 0.05.

3. Results and discussion

3.1. Microbiological analysis

In the course of the 3 months of the study, neither deep freezing at -20 ± 2 °C, nor refrigeration for 48 h after deep freezing altered the sterility of the ophthalmic solutions. No microorganism was isolated on either of the culture media. The thawing procedure (slow at ambient temperature or fast under warm running tap water) did not affect the microbiological quality of the vancomycin ophthalmic solutions. However, the bottles must be airtight. It is therefore necessary to check the condition of the bottles before storage of the ophthalmic solutions.

No difference was observed between readings at 48 h and at 7 days for the trypticase-soy media.

3.2. Visual inspection

Visual inspection of the ophthalmic solutions showed no particles, irrespective of the storage and thawing conditions.

Table 2
Time course of osmolality (on mOsm/kg) of vancomycin ophthalmic solutions (25 mg/ml) as a function of storage time

Time (days)	After deep freezing and rapid thawing	After deep freezing and thawing at ambient temperature	Time (days)	After deep freezing and refrigeration during 48 h
D7	317.3 ± 4.5	321.0 ± 2.9	D9	316.3 ± 5.0
D14	321.7 ± 5.2	319.7 ± 2.6	D16	319.7 ± 1.7
D30	317.0 ± 3.7	315.7 ± 4.6	D32	320.7 ± 6.6
D60	310.0 ± 10.6	318.7 ± 3.3	D62	319.7 ± 5.3
D90	317.7 ± 4.5	321.0 ± 5.4	D92	318.3 ± 1.7

Table 3
Precision of the vancomycin assay method

Concentration added (μg/ml)	Intra-day assay variability ($n = 10$)		Inter-day assay variability ($n = 12$)	
	Concentration found (μg/ml)	Coefficient of variation (%)	Concentration found (μg/ml)	Coefficient of variation (%)
50	48.2 ± 0.1	0.1	50.2 ± 2.4	4.7
100	101.6 ± 0.6	0.6	102.7 ± 3.7	3.6
200	196.3 ± 0.4	0.2	204.1 ± 5.7	2.8
400	405.5 ± 1.1	0.3	405.9 ± 11.5	2.8

3.3. pH

No noteworthy variation of the pH of the vancomycin ophthalmic solutions was observed during the 3 months of the study. It remained stable at between 3.6 and 3.9, a value that is compatible with intraocular administration of the solution. The tear film has a buffering action (pH 7.4) that will bring the pH of solutions between 3.5 and 10.5 into a tolerable range (between 6.4 and 9.6) provided their own buffering capacity is low (Sauvageon-Martre et al., 1993).

3.4. Osmolality

The different values of osmolality of the ophthalmic solutions measured during the 3 months of the study never differed by more than 5% of the average value of the osmolality measured immediately after their preparation (315.7 ± 3.1 mOsm/kg). They remained between 296.8 and 334.6 mOsm/kg, as indicated in Table 2. These values are fully compatible with intraocular administration of the vancomycin 25 mg/ml ophthalmic solution. We observed no significant difference in the osmolality over time for each storage condition ($P = 0.542$ after deep freezing and rapid thawing, $P = 0.551$ after deep freezing and thawing at ambient temperature and $P = 0.925$ after deep freezing and refrigeration during 48 h). Statistical analysis showed no significant influence of the thawing procedure ($P = 0.262$).

3.5. Assay of vancomycin

The method of assay of vancomycin was developed and validated at the Control and Development Laboratory of the Pharmacy. The precision of the method (intra-day and inter-day assay variabilities) was satisfactory with coefficients of variation less than 5% (Table 3). The linearity of the method was satisfactory for vancomycin concentrations of 50–400 μg/ml: the correlation coefficient was 0.9994. The equation of the mean calibration line was $Y = 2.19 \times 10^4 \ X + 1.26 \times 10^5$. The method developed analyses vancomycin specifically and independently of any breakdown products (chromatographic analysis showed that

these did not interfere with the response of the parent compound). The Fig. 1 showed a chromatogram of 25 mg/ml vancomycin ophthalmic solution after a 1/100 dilution.

The results of the stability study are presented in Table 4. We observed no variation in the vancomycin concentration by more than 5% of the initial value on D0 (immediately after preparation of the ophthalmic solutions) during the 3 months of deep freezing at -20 °C.

Vancomycin also remained stable when the ophthalmic solutions were refrigerated for 48 h after deep freezing. No breakdown product was evidenced in any of the samples analysed by high performance liquid chromatography.

We observed no significant difference in the concentration of vancomycin over time for each storage conditions ($P = 0.274$ after deep freezing and rapid thawing, $P = 0.461$ after deep freezing and thawing at ambient temperature and $P = 0.462$ after deep freezing and refrigeration during 48 h).

Likewise, the thawing procedure did not interfere in any way with the physicochemical stability of the ophthalmic solutions. Statistical analysis showed no evidence of any significant influence of the thawing procedure in concentrations of vancomycin ophthalmic solutions ($P = 0.199$).

4. Conclusion

The results of this study fully demonstrate the stability of 'strengthened' ophthalmic solutions of vancomycin 25 mg/ml deep frozen for 3 months at -20 ± 2 °C. This stability was maintained when the ophthalmic solutions were stored for 48 h in a refrigerator after thawing. The thawing procedure had no influence on the stability of vancomycin.

The advantages of deep freezing ophthalmic solutions are many. First it is possible to carry a stock of ophthalmic solutions, making them more promptly available for dispensing to patients by removing the delay due to extemporaneous preparation time. Further, the quality of these solutions is improved relative to extemporaneous preparations because as they are made up in advance in series they can be subjected to physical, chemical and microbiological quality controls (per batch) before administration to patients. Although physical and chemical controls can be carried out on

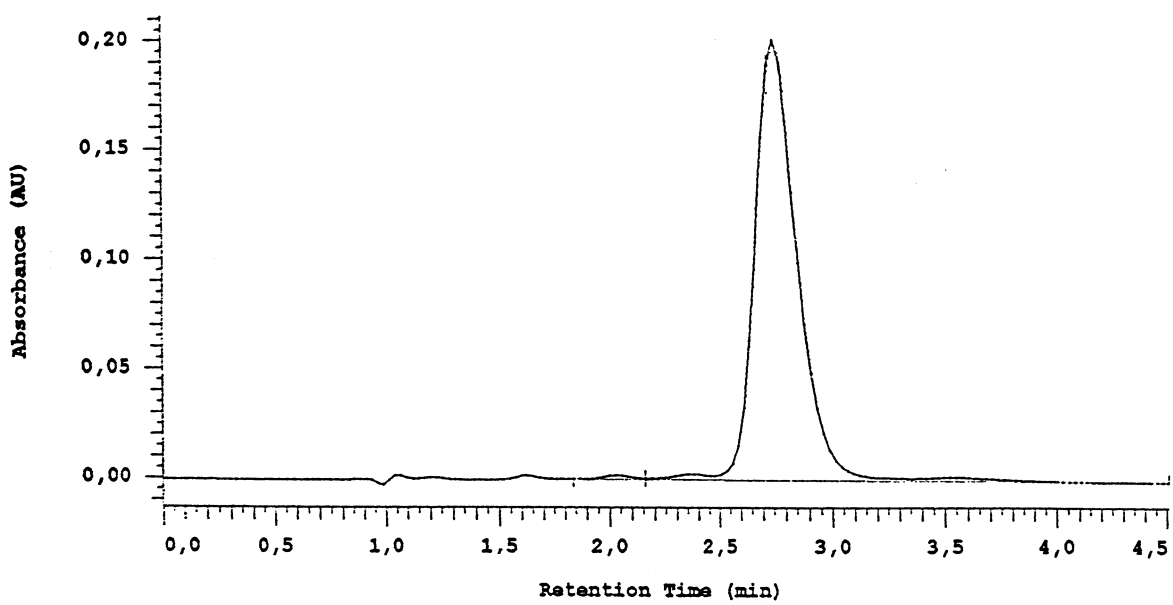

Fig. 1. Chromatogram of a 25 mg/ml vancomycin ophthalmic solution after a 1/100 dilution.

Table 4
Stability of vancomycin (25 mg/ml) after deep freezing and after deep freezing plus refrigerated storage for 48 h

	After deep freezing and rapid thawing		After deep freezing and thawing at ambient temperature			After deep freezing and refrigeration during 48 h	
Time (days)	Concentration C1 (mg/ml)	Percent concentration remaining after storage (%)	Concentration C2 (mg/ml)	Percent concentration remaining after storage (%)	Time (days)	Concentration C3 (mg/ml)	Percent concentration remaining after storage (%)
D0	234.3 ± 5.4	100.0	234.3 ± 5.4	100.0	D2	227.7 ± 3.0	97.1
D7	229.0 ± 2.2	97.7	227.0 ± 0.9	96.9	D9	227.4 ± 1.0	97.0
D14	233.6 ± 2.9	99.7	232.7 ± 4.2	99.3	D16	234.4 ± 5.8	100.0
D30	231.6 ± 2.3	98.8	230.5 ± 2.3	98.4	D32	229.5 ± 11.8	97.9
D60	234.3 ± 4.9	100.0	235.3 ± 4.1	100.4	D62	223.8 ± 2.3	95.5
D90	227.0 ± 2.3	96.9	226.3 ± 1.8	96.6	D92	231.7 ± 3.4	98.9

extemporaneous preparations, the 7 days incubation required for microbiological controls make it impossible to have the results before the preparation is used.

Lastly, storage for 3 months in deep freeze allow better control over production. These ophthalmic solutions can be dispensed to a patient in an eye ward for a 2-day treatment. Like all ophthalmic solutions made up ad hoc they have to be used within 24 h after opening. Obviously, the condition of the container has to be checked before use, because the sterility of the solution is maintained only if the bottle closure is airtight.

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