



HAL
open science

Stability studies of five anti-infectious eye drops under exhaustive storage conditions.

Christophe Curti, E Lamy, N Primas, Cyril Fersing, C Jean, P Bertault-Peres,
P Vanelle

► To cite this version:

Christophe Curti, E Lamy, N Primas, Cyril Fersing, C Jean, et al.. Stability studies of five anti-infectious eye drops under exhaustive storage conditions.. Pharmazie, 2017, 72 (12), pp.741-746. 10.1691/ph.2017.7089 . hal-04547479

HAL Id: hal-04547479

<https://hal.science/hal-04547479>

Submitted on 15 Apr 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Service Central de la Qualité et de l'Information Pharmaceutiques (SCQIP)¹, Assistance Publique - Hôpitaux de Marseille (AP-HM), Hôpital de la Conception; Equipe de Pharmaco-Chimie Radicalaire², Laboratoire de Biochimie³, Faculté de Pharmacie, Aix-Marseille Université; Pharmacie Usage Intérieur Hôpital Sainte-Marguerite⁴, Assistance Publique - Hôpitaux de Marseille (AP-HM), Hôpital Sainte-Marguerite; Pharmacie Usage Intérieur Hôpital Timone⁵, Assistance Publique - Hôpitaux de Marseille (AP-HM), Hôpital Timone, Marseille, France

Stability studies of five anti-infectious eye drops under exhaustive storage conditions

C. CURTI^{1,2,*}, E. LAMY^{1,3}, N. PRIMAS^{1,2}, C. FERSING², C. JEAN⁴, P. BERTAULT-PERES^{4,5}, P. VANELLE^{1,2}

Received May 18, 2017, accepted July 28, 2017

*Corresponding author: Christophe Curti, PhD, PharmD, Assistance Publique - Hôpitaux de Marseille (AP-HM), Service Central de la Qualité et de l'Information Pharmaceutiques (SCQIP), Hôpital de la Conception, 147 Boulevard Baille, 13005 Marseille, France
christophe.curti@univ-amu.fr

Pharmazie 72: 741–746 (2017)

doi: 10.1691/ph.2017.7089

Several ocular infections require anti-infectious eye drops prepared by hospital pharmacy. Stability of these preparations is described in the literature, but studies do not always adequately consider physico-chemical parameters or storage conditions. We describe herein a complete study conducted on five anti-infectious eye drops containing vancomycin, gentamicin, ceftazidime, amphotericin B and voriconazole. We looked for significant changes in active pharmaceutical ingredient content, pH, osmolality and subvisible particles. Our study was designed to monitor stability at ambient temperature, at 2–8 °C, and also at 2–8 °C after various freezing periods. Under ambient storage conditions, eye drops were stable for 15 days, except for ceftazidime, which was stable for less than 1 day only. Under refrigeration conditions (2–8 °C), amphotericin B and voriconazole were stable for 60 days, vancomycin and gentamicin were stable for 30 days while ceftazidime was only stable for 15 days. After 90 days freezing and thawing, voriconazole remained stable at 2–8 °C for 60 days, vancomycin and amphotericin B for 30 days and gentamicin only for 21 days. Ceftazidime eye drops were stable for only 7 days at 2–8 °C after 60 days freezing. Our results are compared to the most relevant publications. Results of this study allow the compounding of large batches of harmonized anti-infectious eye drops.

1. Introduction

Several anti-infectious ophthalmic solutions can be administered as a treatment for bacterial or fungal keratitis. For bacterial keratitis, vancomycin (Cahane et al. 2004; Chiquet and Romanet 2003; Bourcier et al. 2003) and gentamicin (Gokhale 2008; Chiquet and Romanet 2003; Bourcier et al. 2003) are frequently used. While still commercially available, ticarcillin (Bourcier et al. 2003) was also widely prescribed, but can currently be replaced by ceftazidime (Wijesooriya et al. 2013; Hue et al. 2009). Voriconazole (Al-Badriyeh et al. 2010) and amphotericin B (Wood et al. 1985) eye drops can be used for the treatment of fungal keratitis. Topical anti-infectious eye drops treatment must be compounded with high concentration to enhance efficacy with constant innocuity. All these molecules except gentamicin are not currently commercially available in a galenic formulation compatible with ophthalmic administration. Thus, vancomycin, ceftazidime, amphotericin B and voriconazole are only available as prepared by pharmacy staff from parenteral anti-infectious drugs. Pharmaceutical preparations of fortified gentamicin eye drops (generally 1.4%) can also be prescribed for keratitis treatment.

The literature contains several stability studies conducted on one of these drugs, but these studies often lack one or more important parameter. For ophthalmic preparations, the European Pharmacopoeia (EP 2017) recommends the evaluation of the concentration of the drug, pH, osmolality and subvisible particles and the United States Pharmacopoeia plans revisions to include these tests (USP 2017). Moreover, eye drop stability should be studied under three different conditions: 2–8 °C (classical storage conditions), –20 °C (for preparation of large batches and/or due to high cost of drug used for preparation) and 25 °C (if patient or nurse omitted to put eye drops in the fridge). Here, we describe a stability study of five

anti-infectious eye drops under these three storage conditions that considers the above parameters.

2. Investigations and results

2.1. HPLC analytical conditions

The anti-infectious drug content of the solution was determined by HPLC. Previously described methods were used for gentamicin (Kim et al. 2003), amphotericin B (Peyron et al. 1999) and voriconazole (Tang 2013). Based on a previously described method, the gradient mode was changed to an isocratic mode and another column was used for vancomycin analysis (Ghassem-pour et al. 2001). The method described for the quantification of ticarcillin was used with minor modifications (gradient of mobile phase, column) for ceftazidime (Galvez et al. 2007). Parameters are given in section 4.1. Injection volumes for each anti-infectious sample were fixed at 20 µL.

Gentamicin samples were subjected to derivatization, as described in the literature (Kim et al. 2003, 2001). TEA stock solutions in ACN (100 µL of TEA in 14.6 mL of ACN) can be prepared and stocked for six months at 2–8 °C. Phenylisocyanate reagent (50 µL of phenylisocyanate in 10 mL of ACN) must be prepared extemporaneously and remained stable only 24 h at 2–8 °C. After opening, commercial phenylisocyanate reagent in a sealed glass container and under inert atmosphere remained stable for six months at –20 °C. For derivatization, 500 µL of sample, 250 µL of TEA solution and 250 µL of phenylisocyanate solution were mixed, vortexed 5 min and heated 15 min in an oven at 50 °C. As reported (Kim et al. 2003, 2001), a mixture of five structurally related components (gentamicin C1a, C2a, C2b, C1 and C2), appeared as five peaks on the chromatogram after derivatization. Several peaks resulting

Table 1: Retention time and linearity

Samples	Retention time	Linearity	Calibration line ($\mu\text{g/mL}$)	R^2
Vancomycin	8.0 min	0 - 40 $\mu\text{g/mL}$	2.5-5-10-20-40	0.9999
Gentamicin	17.5-27 min	0 - 1000 $\mu\text{g/mL}$	62.5-125-250-500-1000	0.9997
Ceftazidime	16.9 min	0 - 1000 $\mu\text{g/mL}$	100-200-400-600-800-1000	0.9995
Amphotericin B	2.5 min	0 - 2 $\mu\text{g/mL}$	0.25-0.5-0.75-1-2	0.9994
Voriconazole	16.7 min	0 - 500 $\mu\text{g/mL}$	10-50-100-200-500	0.9999

from phenylisocyanate excess or its degradation products (retention time < 5 min) were also observed. Moreover, five small peaks (retention times between 6.50 and 10.77 min) were noticed and could be due to gentamicin impurities as they are very similar to the five peaks of gentamicin.

Linearity graphs were obtained from the peak area of every calibration point. Regression coefficients R^2 were calculated, showing good linearity and a linear response in the chosen concentration range. Results are summarized in Table 1, and typical chromatograms were shown in Fig. 1.

The following parameters were evaluated in the method validations: selectivity, specificity, linearity, repeatability (within-day variation), intermediate precision (between-day variation) and accuracy.

Within-day measurements were carried out at two concentrations in both cases, with at least ten samples on the same day. After each

analysis, the chromatogram was integrated and the concentration was calculated: the percentage of relative standard deviation (% RSD) was found to be less than 2, indicating the good precision of the methods. Between-day measurements were carried out at the same concentrations, with at least 20 samples on at least three days. The results for repeatability, intermediate precision and accuracy are summarized in Table 2.

2.2. Stability study design

For each anti-infectious drug and each storage condition, a bibliographic survey was performed to determine the best stability study procedure. We also took into account the expected frequency of batch production in order to rationalize costs.

Under ambient storage conditions, vancomycin was studied for 30 days, gentamicin, amphotericin B and voriconazole were studied

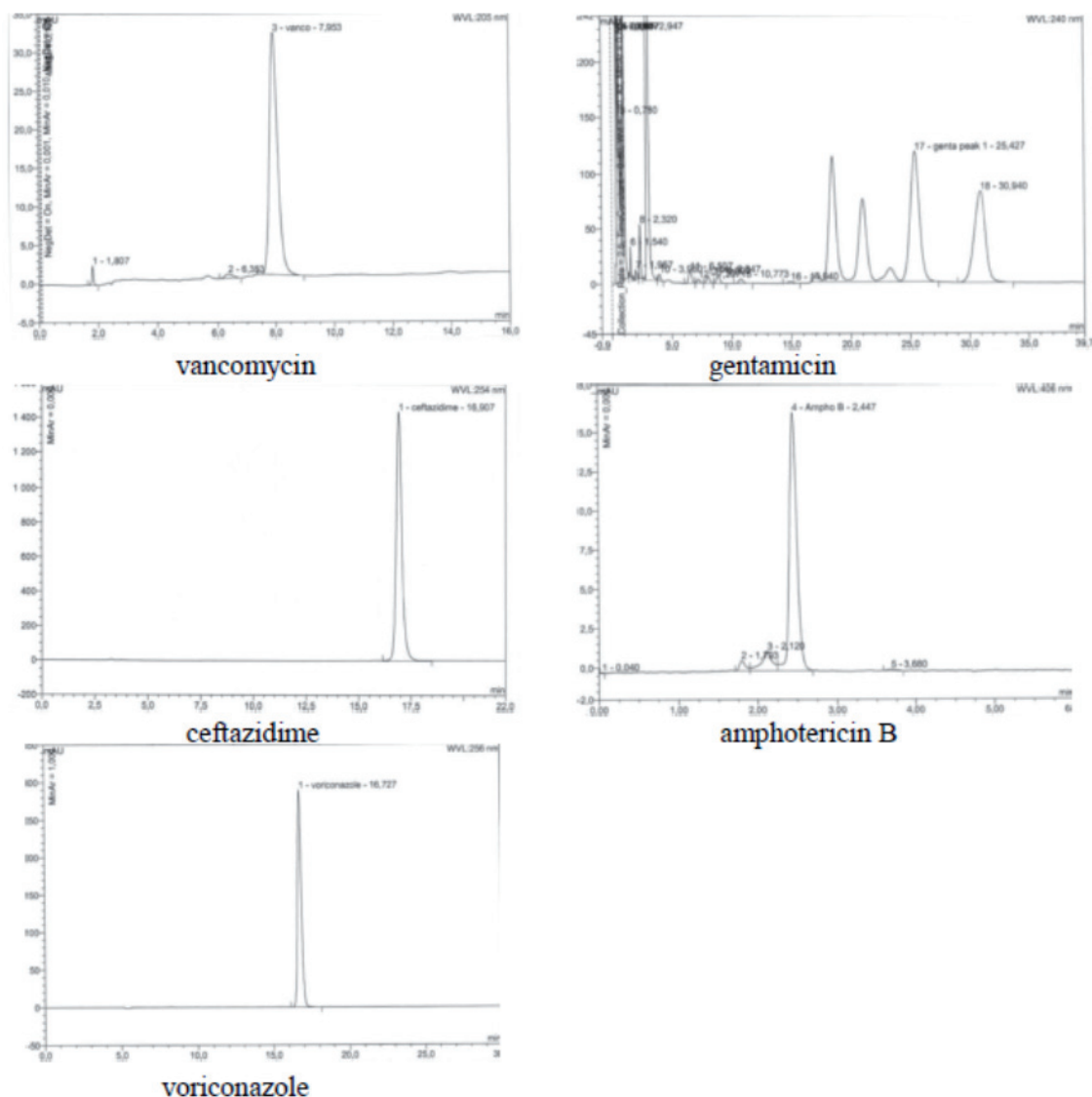


Fig. 1: Chromatograms of anti-infectious drugs

Table 2: Repeatability, intermediate precision and accuracy

Samples (Concentration levels, µg/mL)	% RSD within-day		% RSD between-day			Bias
Vancomycin (10 / 7.5)	4.61%	1.91%	3.89%	1.69%	2.40%	- 0.50%
Gentamicin ^a (250 / 500)	6.17%	3.82%	5.39%	4.67%	0.94%	- 1.49%
Ceftazidime (200 / 500)	0.18%	0.52%	0.46%	1.18%	2.60%	0.50%
Amphotericin B (1 / 0.75)	2.15%	1.70%	2.35%	3.84%	0.59%	- 2.60%
Voriconazole (100 / 200)	0.62%	0.35%	1.65%	4.95%	- 4.24%	- 3.05%

^a method validation for gentamicin was realized with total peak area of the four first peaks of derivatized gentamicin.

for 15 days, and ceftazidime only for 2 days. Under refrigeration storage conditions (2-8 °C), vancomycin and gentamicin were studied for 30 days, amphotericin B and voriconazole for 60 days, and ceftazidime for 21 days. Finally, after 3 months freezing (-20 °C), vancomycin and gentamicin were studied under refrigeration storage conditions (2-8 °C) for 30 days, amphotericin B and voriconazole for 60 days and ceftazidime for 15 days. For each parameter, analyses were performed in triplicate at each point of measurement.

The International Conference on Harmonization defined significant changes for stability studies as “a 5% change in assay from its initial value”. These limitations are applicable to new drug substances in pharmaceutical industry, but can be discussed for hospital preparations. Stability studies for anticancer drugs or active pharmaceutical ingredients (API) with narrow therapeutic index should have the same limits than defined by ICH (Bardin et al. 2011). For other types of compounded preparations, stability limits were frequently defined in the literature as a 10% change in assay from its initial value (Chennell et al. 2017; Ezquer-Garin et al. 2017; Friciu et al. 2017; Dreno et al. 2015). Therefore, in the present study, limits were also considered as a 10% change in assay from its initial value (drug content, pH, osmolality) or as a failure to meet its acceptance criterion (subvisible particles).

2.3. Results

Initial physico-chemical parameters are reported in Table 3.

Table 3: Physico-chemical parameters of studied eye drops

	Formulation	pH (+/- 10%)	Osmolality (+/- 10%) mOsm/kg
Vancomycin	50 mg/mL, NaCl 0.9%	3.11 (2.80 - 3.42)	335 (301-368)
Gentamicin	14 mg/mL, NaCl 0.9%	3.96 (3.56 - 4.36)	303 (272-333)
Ceftazidime	20 mg/mL, NaCl 0.9%	6.99 (6.29 - 7.69)	359 (323-395)
Amphotericin B	5 mg/mL, G5%	7.46 (6.71 - 8.21)	364 (328-400)
Voriconazole	10 mg/mL, water for injection	6.37 (5.73 - 7.00)	513 (462-564)

Physico-chemical parameters remained above stability limits (> 10% change from its initial value for pH and osmolality and failure to meet acceptance criterion for subvisible particles) for any storage condition and for any studied molecule. However several decreases in content (> 10%) of the pharmaceutical products were observed as reported in Figs. 2, 3 and 4.

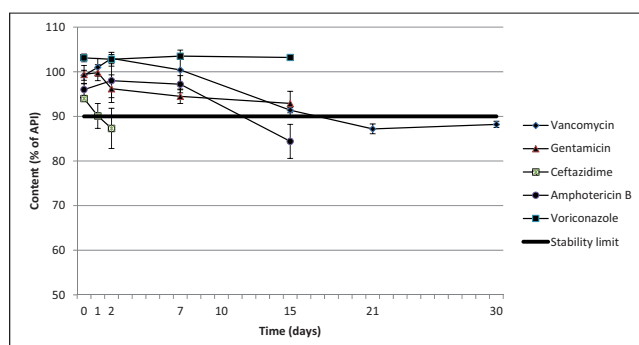


Fig. 2 Ambient temperature stability of anti-infectious eye drops

Under ambient storage conditions, vancomycin, gentamicin, amphotericin B and voriconazole eye drops were stable for 15 days (Fig. 2). As expected, ceftazidime content quickly decreased, and ceftazidime eye drops were stable for less than 1 day.

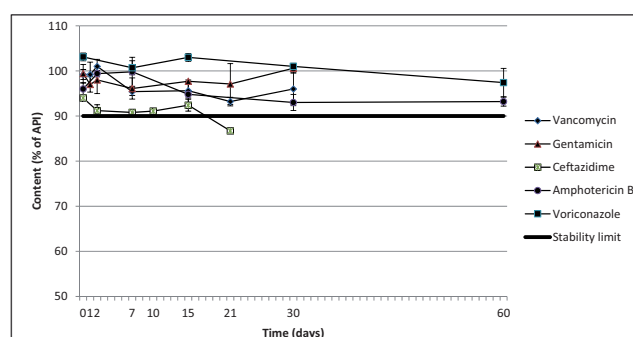


Fig. 3: 2-8 °C stability of anti-infectious eye drops

Under refrigeration conditions (2-8 °C), amphotericin B and voriconazole were stable for 60 days, vancomycin and gentamicin were stable for 30 days while ceftazidime was only stable for 15 days (Fig. 3).

After 90 days freezing and thawing, voriconazole remained stable at 2-8 °C for 60 days, vancomycin and amphotericin B for 30 days and gentamicin only for 21 days (Fig. 4). Since content of

ceftazidime eye drops after 90 days freezing quickly decreased, we repeated the stability study after 30 days and 60 days freezing. Stability after 60 days freezing is reported in Fig. 5, showing that ceftazidime eye drops were stable for only 7 days at 2-8 °C after 60 days freezing. Similar results were obtained after 30 days freezing.

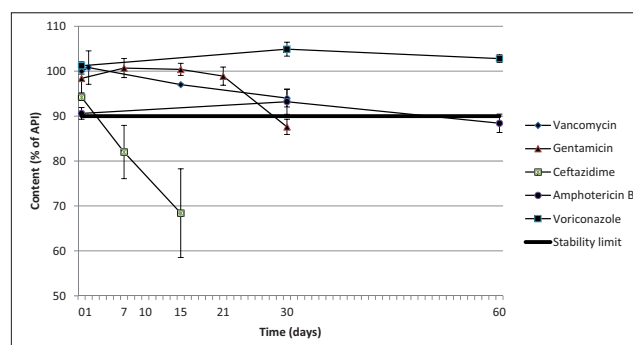


Fig. 4: 2-8 °C stability of anti-infectious eye drops after 90 days freezing

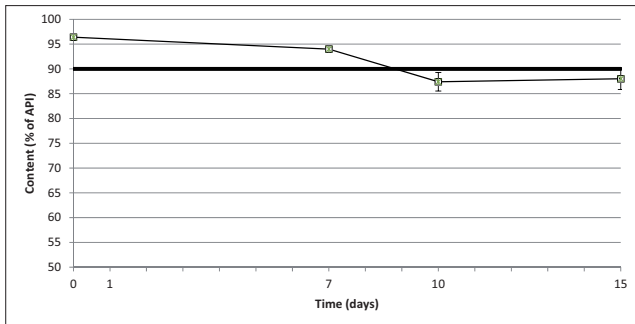


Fig. 5: 2-8 °C stability of ceftazidime eye drops after 60 days freezing

Analysis between different times was performed with a non-parametric Friedman test. Significant differences were found, even if the limit values (10%) were not reached. Significant differences were found for vancomycin ($p < 0.02$) and gentamicin ($p < 0.05$) under ambient storage conditions, for each drug ($p < 0.05$) except for gentamicin for 2-8 °C storage conditions and except for gentamicin and vancomycin for thawing storage conditions. Moreover, a significant decrease of the slope of the regression line was observed for vancomycin ($p < 0.001$) and gentamicin ($p < 0.02$) under ambient storage conditions, and for vancomycin ($p < 0.001$) and ceftazidime ($p < 0.02$) for 90 days thawing storage conditions. This statistical analysis emphasized the importance of continuing analyses during stability study even if the limit value is reached, as the lack of data (for example for amphotericin B and ceftazidime under ambient storage condition in our study) can be damaging for statistical analysis.

3. Discussion

Results obtained in this study were compared to the literature for each studied molecule and reported in Tables 4-8. Different studies in the literature lack at least one of our storage temperature parameters. Our work is the first example of stability studied for five anti-infectious eye drops under three different storage conditions. The stability of 50 mg/mL vancomycin eye drops in NaCl 0.6% found in our study was close to the best results from several studies (Table 4). Moreover, after 90 days at -20 °C, vancomycin eye drops can be stocked for 30 days at 2-8 °C. To our knowledge, this parameter has never been studied. Our results suggest that hospital pharmacists can produce larger batches of vancomycin eye drops, with the resulting cost-effective rationalization of production time and volume.

The stability of 14 mg/mL gentamicin eye drops in NaCl 0.9% at ambient temperature was found here to be higher than the stability

previously reported for the same formulation (Table 5) (Ho et al. 2001). We found that gentamicin remained stable after 30 days at 2-8 °C, but also that 90 days freezing at -20 °C decreased its stability at 2-8 °C to 21 days only. To our knowledge, this parameter has never been studied, and, as reported for vancomycin, our results suggest that larger batches can be realized.

Several studies described the use of Sno Tears® for the compounding of viscous ceftazidime eye drops (Barnes and Nash 1999; Barnes 1995). As other drugs, Sno Tears® is not commercialized worldwide, and could be replaced if not available. Such artificial tears solutions contains several excipients/active pharmaceutical ingredients (for example, Sno Tears®: benzalkonium chloride, disodium edetate, hydroxyethylcellulose, polyvinyl alcohol...) increasing the risk of physico-chemical incompatibilities with ceftazidime. Taking these points into consideration, we preferred to test the use of a worldwide available solvent: NaCl 0.9%.

Ceftazidime eye drops 20 mg/mL in NaCl 0.9% were found to be very unstable (Table 6). Under ambient temperature, stability appeared to be less than one day, contrary to findings from previous studies. Therefore, it is very difficult to apply an expiration date to eye drops left outside the fridge, since temperatures in hospital wards or patients' homes vary more than in the laboratories where stability studies are carried out. The stability of frozen ceftazidime eye drops was found to be shorter than previously reported in the literature (60 days versus 75 days (Chédu-Legros et al. 2010)). Moreover, our findings imply that eye drops can only be stocked for 7 days at 2-8 °C after thawing.

Amphotericin B is incompatible with NaCl 0.9%, (WHO 1999) and must therefore be solubilized with 5% glucose or with water for injection. The stability of 5 mg/mL amphotericin B eye drops in 5% glucose was found to be shorter than reported in the literature (Peyron et al. 1999) at ambient temperature (Table 7). Moreover, although stability for as long as 120 days at 2-8 °C has been reported, we only studied stability over 60 days at 2-8 °C. We also found only 30 days stability after 90 days at -20 °C.

Voriconazole eye drop preparations are made from the commercial parenteral voriconazole. This formulation contains cyclodextrin as excipient, leading to hyperosmolar eye drop formulations (Dupuis et al. 2009). Therefore, in order to decrease osmolality, water for injection must be used rather than NaCl 0.9%. Voriconazole eye drops 10 mg/mL were reported, both in the literature and in our study, to be very stable, whatever the temperature is (Table 8). Our study found that voriconazole can be frozen for 90 days and still remains stable for 60 days at 2-8 °C after thawing. When the cost of parenteral voriconazole is taken into consideration, these results justify the production of large batches, with less risk of discarding expired eye drops.

Compounding of studied eye drops were realized with NaCl 0.9% (vancomycin, gentamicin, ceftazidime), G5% (amphotericin B) or

Table 4: Vancomycin eye drop stability

Reference	Formulation	Ambient temperature	2-8 °C	-20 °C/2-8 °C
Sautou-Miranda et al. (2002)	25 mg/mL, G5%			90 days / n.d.
Barbault et al. (1999)	50 mg/mL, NaCl 0.9%	15 days	21 days	
Fuhrman and Stroman (1998)	31 mg/mL, HMPC/ Dextran/ benzalkonium/ EDTA/water	7 days	10 days	45 days/ n.d.
Chédu-Legros et al. (2010)	50 mg/mL, G5%		28 days	75 days/ n.d.
Chédu-Legros et al. (2010)	25 mg/mL, NaCl 0.9%			30 days/ n.d.
Present study	50 mg/mL, NaCl 0.9%	15 days	30 days	90 days/30 days

Table 5: Gentamicin eye drop stability

Reference	Formulation	Ambient temperature	2-8 °C	-20 °C/2-8 °C
Ho et al. (2001)	8 mg/mL, NaCl 0.9%	7 days	42 days	
Ho et al. (2001)	14 mg/mL, NaCl 0.9%	7 days	42 days	
McBride et al. (1991)	13.6 mg/mL		90 days	
Arici et al. (1999)	13.5 mg/mL, commercial eye drops mixed with commercial parenteral form	21 days (antimicrobial activity)	21 days (antimicrobial activity)	
Present study	14 mg/mL, NaCl 0.9%	15 days	30 days	90 days/21 days

Table 6: Ceftazidime eye drop stability

Reference	Formulation	Ambient temperature	2-8 °C	-20 °C/2-8 °C
Barnes (1995)	50 mg/mL, Snotears®		7 days	
Barnes and Nash (1999)	50 mg/mL, Snotears®	1 day	5 days	
Kodym et al. (2011)	50 mg/mL, citrate buffer	2 days	12 days	
Chédu-Legros et al. (2010)	50 mg/mL, NaCl 0.9%			75 days
Present study	20 mg/mL, NaCl 0.9%	Less than 1 day	15 days	60 days/7 days

Table 7: Amphotericin B eye drop stability

Reference	Formulation	Ambient temperature	2-8 °C	-20 °C/2-8 °C
Peyron et al. (1999)	5 mg/mL, G5%	13-16 days ^a	120 days	
Dermu et al. (2015)	5 mg/mL, G5%/sodium bicarbonates			60 days/4 days
Present study	5 mg/mL, G5%	7 days	60 days	90 days/30 days

^a Under different storage conditions: 13 days under exposure to light and 16 days without light

Table 8: Voriconazole eye drop stability

Reference	Formulation	Ambient temperature	2-8 °C	-20 °C/2-8 °C
Dupuis et al. (2009)	10 mg/mL, water for injection	30 days	30 days	
Amorós-Reboredo et al. (2015)	10 mg/mL, water for injection			90 days/14 days
Al-Badriyeh et al. (2009)	10 mg/mL, water for injection, benzalkonium chloride		98 days	
Al-Badriyeh et al. (2009)	20 mg/mL, water for injection, benzalkonium chloride	112 days	112 days	
Present study	10 mg/mL, water for injection	15 days	60 days	90 days/60 days

water for injection (voriconazole), and are therefore easily reproducible worldwide. We assume that eye drops were unbuffered in order to avoid unforeseen physico-chemical incompatibilities, such as the recently described incompatibility between amphotericin B/bicarbonates in mouthrinses (Jolivot et al. 2012) as this association was also described in eye drops formulation (Dermu et al. 2015). Since 2014, we dispensed these eye drops to many patients, without any report of undesirable effect. The number of dispensed eye drops since 2014 is summarized in Table 9.

Table 9: Dispensed eye drops since 2014

	2014	2015	2016	2017	Total
Vancomycin	471	360	367	129	1327
Gentamicin	-	256	418	128	802
Ceftazidime	-	-	140	59	199
Amphotericin B	-	-	25	23	48
Voriconazole	-	-	86	42	128

We described comprehensive stability studies on five anti-infectious eye drops. Our results were close to those reported in the literature, with the advantage of compiling comprehensive data within one study. Contrary to most previously described studies, the full range of recommended physico-chemical parameters and storage conditions were studied. Our findings should encourage

the pharmaceutical compounding of large batches of harmonized eye drops, with beneficial effects for clinicians, patients and hospital costs.

4. Experimental

4.1. Instrumentation

pH was determined with a Thermo Scientific® Orion 4 Star pH-meter, calibrated with Radiometer Analytical® standard etalons (pH 4.005, pH 7.000 and pH 10.012). Osmolality was determined with Löser® manual osmometer type 6, calibrated with 300 and 900 mOsm/kg standard solutions. Sub-visible particles were determined under a Zeiss optical microscope at X10, with Kova® slide. Standardized 1.0% polystyrene microsphere suspensions in water (Polysciences®), of 25 µm and 50 µm diameter, were used as references for determination of particle size. Analytical conditions are given in Table 10.

Chromatography was carried out on an automatic high performance liquid chromatography Dionex® Ultimate 3000 with a UV diode array detector. The set-up was connected to an HP 1702 computer equipped with chromatographic data processing software (Chromeleon Chromatography Management System, Version 6.80 SRH Biold 3161, 1994-2011 Dionex Corporation).

4.2. Subvisible particles experimental conditions

According to the European Pharmacopeia, a volume corresponding to 10 µg of active pharmaceutical ingredient (this volume was calculated for each eye drops: vancomycin: 0.2 µL, gentamicin: 0.896 µL, ceftazidime: 0.5 µL, amphotericin B: 2 µL and voriconazole: 1 µL) must contain less than 20 particles of any size greater than 25 µm, less than 2 particles greater than 50 µm and no particles greater than 90 µm. Each

Table 10: HPLC conditions

Samples	Columns	Mobile phases	λ
Vancomycin	Nucleosil C18, 5 µm, 4.6 X 250 mm	ACN / THF / 0.1% TEA in water adjusted to pH 6.2 with phosphoric acid (9/1/90), 1.5 mL/min	205 nm
Gentamicin	Capcell Pak C18, 3 µm, 4.6 X 50 mm	ACN with 0.1% TFA / water (40/60), 1.5 mL/min	240 nm
Ceftazidime	Lichrospher C18, 5 µm, 4.6 X 250 mm	Phase A: phosphate buffer pH 5 Phase B: mobile phase A/methanol (66/34), gradient T0 min 100 % A, T20 min 45%A/55%B, 0.8 mL/min	254 nm
Amphotericin B	Kromasil C18, 5 µm, 4.6 X 250 mm	ACN / water with 1% acetic acid (46/54), 1 mL/min	406 nm
Voriconazole	Nucleosil C18, 5 µm, 4.6 X 250 mm	ACN / methanol / 0.05 M ammonium acetate in water (20/40/40), 0.5 mL/min	256 nm

Table 11: Subvisible particles limit values in anti-infectious eye drops

Sample	Volume (µL) corresponding to 10 µg	Kova® slide grids to count	Particles > 25 µm	Particles > 50 µm	Particles > 90 µm
Vancomycin 50 mg/mL	0.2	1	100	10	0
Gentamicin 14 mg/mL	0.896	1	22	2	0
Ceftazidime 20 mg/mL	0.5	1	40	4	0
Amphotericin B 5 mg/mL	2	2	20	2	0
Voriconazole 10 mg/mL	1	1	20	2	0

Kova® slide counting grid representing a volume of 1 µL, these limit values were transposed for a known volume of 1 or 2 µL, as summarized in Table 11.

Conflicts of interest: All Authors declare that they have no conflicts of interest.

References

- Al-Badriyeh D, Li J, Stewart K, Kong DC, Leung L, Davies GE, Fullinlaw R (2009) Stability of extemporaneously prepared voriconazole ophthalmic solution. *Am J Health-Syst Pharm* 66: 1478 - 1483.
- Al-Badriyeh D, Fen Neoh C, Stewart K, Kong DCM (2010) Clinical utility of voriconazole eye drops in ophthalmic fungal keratitis. *Clin Ophthalmol* 4: 391–405.
- Amorós-Reboredo P, Bastida-Fernandez C, Guerrero-Molina L, Soy-Muner D, López-Cabezas C (2015) Stability of frozen 1% voriconazole ophthalmic solution. *Am J Health-Syst Pharm* 72: 479-482.
- Arici MK, Sümer Z, Güler C, Elibol O, Saygi G, Cetinkaya S (1999) In vitro potency and stability of fortified ophthalmic antibiotics. *Aust N Z J Ophthalmol* 27: 426-30.
- Barbault S, Aymard G, Feldman D, Pointereau-Bellanger A, Thuillier A (1999) Stability of vancomycin eye drops. *J Pharm Clin* 18: 183-189.
- Bardin C, Astier A, Vulto A, Sewell G, Vigneron J, Trittler R, Daouphars M, Paul M, Trojniak M, Pinguet F (2011) Guidelines for the practical stability studies of anticancer drugs: a European consensus conference. *Ann Pharm Fr* 69: 221-231.
- Barnes AR (1995) Determination of ceftazidime and pyridine by HPLC: application to a viscous eye drop formulation. *J Liquid Chrom* 18: 3117-3128.
- Barnes AR, Nash S (1999) Stability of ceftazidime in a viscous eye drop formulation. *J Clin Pharm Ther* 24: 299-302.
- Bourcier T, Thomas F, Borderie V, Chaumeil C, Laroche L (2003) Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. *Br J Ophthalmol* 87: 834-838.
- Cahane M, Ben Simon GJ, Barequet IS, Grinbaum A, Diamanstein-Weiss L, Goller O, Rubinstein E, Avni I (2004) Human corneal stromal tissue concentration after consecutive doses of topically applied 3.3% vancomycin. *Br J Ophthalmol* 88: 22–24.
- Chédru-Legros V, Fines-Guyon M, Chérel A, Perdriel A, Albessard F, Debruyne D, Mouriaux F (2010) In vitro stability of fortified ophthalmic antibiotics stored at -20 degrees C for 6 months. *Cornea* 29: 7-11.
- Chennell P, Delaborde L, Wasiak M, Jouannet M, Feschet-Chassot E, Chiambaretta F, Sautou V (2017) Stability of an ophthalmic micellar formulation of cyclosporine A in unopened multidose eyedroppers and in simulated use conditions. *Eur J Pharm Sci* 100: 230-237.
- Chiquet C, Romanet JP (2007) [Prescribing fortified eye drops]. *J Fr Ophtalmol* 30: 423-430. [article in French]
- Dermu M, Lesourd F, Grignon C, Dupuis A (2015) Stability of frozen amphotericin B eye drop [communication]. *IntJClinPharm* 37: 198.
- Dreno C, Gicquel T, Harry M, Tribut O, Aubin F, Brandhonneur N, Dollo G (2015) Formulation and stability study of a pediatric 2% phenylephrine hydrochloride eye drop solution. *Ann Pharm Fr* 73: 31-36.
- Dupuis A, Tournier N, Le Moal G, Venisse V (2009) Preparation and Stability of Voriconazole Eye Drop Solution. *Antimicrob Agents Chemother* 53: 798–799.
- EP – Council of Europe (2017), European Pharmacopeia 9th Ed., “ophthalmic preparations”.
- Ezquer-Garin C, Ferriols-Lisart R, Alós-Almiñana M (2017) Stability of tacrolimus ophthalmic solution. *Am J Health Syst Pharm* 74: 1002-1006.
- Friciu M, Roullin VG, Leclair G (2017) Stability of gabapentin in extemporaneously compounded oral suspensions. *PlosONE* 12: e0175208.
- Fuhrman LC Jr, Stroman RT (1998) Stability of vancomycin in an extemporaneously compounded ophthalmic solution. *Am J Health-Syst Pharm* 55: 1386-1388.
- Galvez O, Mullot JU, Mullot H, Simon L, Grippi R, Payen C, Bonneau D, Huart B, Gentes P (2007) Stability of a 6 mg/mL ticarcillin ophthalmic solution. *Le Pharmacien Hospitalier et Clinicien* 42: 171-176.
- Ghassempour A, Darbandi MK, Asghari FS (2001) Comparison of pyrolysis-mass spectrometry with high performance liquid chromatography for the analysis of vancomycin in serum. *Talanta* 55: 573-80.
- Gokhale NS (2008) Medical management approach to infectious keratitis. *Indian J Ophthalmol* 56: 215–220.
- Ho PC, Soh H, Lim SM, Yow KL (2001) Stability of extemporaneously prepared gentamicin ophthalmic solutions. *Ann Pharmacother* 35: 1293-1294.
- Hue B, Doat M, Renard G, Brandely ML, Chast F (2009) Severe Keratitis Caused by *Pseudomonas aeruginosa* successfully Treated with Ceftazidime Associated with Acetazolamide. *J Ophthalmol*: 794935.
- Jolivot PA, Dunyach-Remy C, Roussey A, Jalabert A, Mallié M, Hansel-Esteller S (2012) [Assessment of in vitro activity and stability of antifungal suspensions for mouthrinses: To a reappraisal of empiric practices?] *Pathologie Biologie* 60: 362-368. [article in French]
- Kim BH, Kim YK, Ok JH (2001) Development of liquid chromatographic method for the analysis of kanamycin residues in varicella vaccine using phenylisocyanate as a derivatization reagent. *J Chromatogr B Biomed Sci Appl* 752: 173-7.
- Kim BH, Lee SC, Lee HJ, Ok JH (2003) Reversed-phase liquid chromatographic method for the analysis of aminoglycoside antibiotics using pre-column derivatization with phenylisocyanate. *Biomed Chromatogr* 17: 396-403.
- Kodym A, Hapka-Zmich D, Golab M, Gwizdala M (2011) Stability of ceftazidime in 1% and 5% buffered eye drops determined by HPLC method. *Acta Pol Pharm* 68: 99-107.
- McBride HA, Martinez DR, Trang JM, Lander RD, Helms HA (1991) Stability of gentamicin sulfate and tobramycin sulfate in extemporaneously prepared ophthalmic solutions at 8 degrees C. *Am J Hosp Pharm* 48: 507-509.
- Peyron F, Elias R, Ibrahim E, Amirat-Combralier V, Bues-Charbit M, Balansard G (1999) Stability of amphotericin B in 5% dextrose ophthalmic solution. *Int J Pharm Compd* 3: 316-20.
- Sautou-Miranda V, Libert F, Grand-Boyer A, Gellis C, Chopineau J (2002) Impact of deep freezing on the stability of 25 mg/ml vancomycin ophthalmic solutions. *Int J Pharm* 234: 1-2.
- Tang PH. Quantification of Antifungal Drug Voriconazole in Serum and Plasma by HPLC-UV (2013) *J Drug Metab Toxicol* 4: 144.
- USP, United States Pharmacopeia (2017), “Ophthalmic Preparations” <771>, discussed in Pharmacopeial Forum (PF) 39(5) [September–October 2013].
- WHO, World Health Organization (1999) Drugs used in HIV-related infections, Geneva, <http://apps.who.int/medicinedocs/en/d/Js2215e/> accessed 20/02/17.
- Wijesooriya C, Budai M, Budai L, Szilasi ME, Petrikovics I (2013) Optimization of liposomal encapsulation for ceftazidime for developing a potential eye drop formulation. *J Basic Clin Pharm* 4: 73–75.
- Wood TO, Tuberville AW, Monnett R (1985) Keratomycosis and amphotericin B. *Trans Am Ophthalmol Soc* 83: 397–409.