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REVIEW

Considerations related to the in-use stability of sterile pharmaceutical dosage forms

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Abstract

Background It is essential to ensure the in-use stability of sterile preparations because they may be vulnerable to microbial contamination during the opening and preparation process. Although some regulatory agencies provide general guidelines for in-use stability, there are no detailed guidelines related to in-use stability testing specific to sterile pharmaceutical dosage forms.

Area covered The guidelines of regulatory authorities and health-care associations related to the in-use stability of sterile pharmaceutical dosage forms were investigated. In addition, research trends on the in-use stability for each dosage form were reviewed.

Expert opinion As a result of the study, we suggest that specific considerations of in-use stability by detailed sterile pharmaceutical dosage form include storage conditions, test period, test interval, withdrawal method, and test measurements.

Introduction

According to the "General Requirements for Pharmaceutical Preparations of the Korean Pharmacopoeia (12th edition)," injections are sterile preparations to be administered directly into the body through the skin, muscle, or blood vessel, usually in the form of solutions, suspensions, or emulsions of drugs substances, or of a solid that contains drugs substances to be dissolved or suspended before use. Unless otherwise specified, sufficient amounts of suitable preservatives to prevent the growth of microorganisms are added to injections in multiple-dose containers (MFDS, 2022).

Injections must be manufactured aseptically in accordance with relevant regulations to minimize the risk of contamination by microorganisms, particulates, and pyrogens (PICS, 2022). However, these preparations may be opened before administration and used for multiple uses according to the dosage approved for the clinical use environment, or reconstituted or diluted due to the nature of the preparation. In such cases, contamination by microorganisms may be involved during the opening, reconstitution, or dilution process, and hence, the in-use stability should be ensured. In a study by Nogler-Semenitz et al. (2007), multidose vials, single-dose vials, and vials containing self-prepared admixtures were collected from various wards to analyze the sterility of their contents during a 4-month period. The authors found that some of the admixtures were contaminated with spore-forming bacteria or coagulase-negative *Staphylococci* and emphasized that a training program is needed for

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health-care workers in aseptic techniques and for validation of the preparation of solutions for parenteral use.

According to the "General Requirements for Pharmaceutical Preparations of the Korean Pharmacopoeia (12th edition)," ophthalmic solutions are sterile preparations of liquid, or solid to be dissolved or suspended before use, intended for application to the conjunctival sac or other ocular tissues. Sufficient amounts of appropriate preservatives to prevent the growth of microorganisms may be added to the preparations filling multiple-dose containers (MFDS, 2022). In a previous study, a total of 200 eye drops were taken from the outpatient departments of Farabi Eye Hospital (Tehran, Iran) after 1, 2, 4, and 7 days of use, and the contamination rates of the residual contents, caps and droppers were determined using conventional techniques (Fazeli et al. 2004). High frequencies of microbial contamination according to the time were detected in the caps and the residual contents of the eye drops. The authors recommended that single-dose drops be used in patients with eye infections. Alternatively, multidose bottles could be discarded after use in an infected patient. Meanwhile, nursing staff should be well trained to handle and administer ophthalmic preparations carefully.

There is still a lack of scientific guidance on how to test the stability of multi-use sterile preparations due to microbial contamination. Lee et al. (2022) noted that the European Medicines Agency (EMA) (EMA, 2001) and Ministry of Food and Drug Safety (MFDS) (MFDS, 2016) have provided guidelines for basic consideration when designing in-use stability tests to set the in-use shelf life relating to nonsterile dosage forms. However, examples of simulation design covering various formulations were not presented in detail. Furthermore, authors have emphasized that it is necessary to establish guidelines for the safe use of drugs on in-use stability for health-care professionals. Choi et al. (2022) explained that appropriate experimental studies should be conducted to support the in-use stability of nonsterile formulations, especially water-containing formulations. However, no specific test methods are currently presented in the relevant guidelines, even though it is necessary to perform specific in-use shelf life designs about the in-use stability of nonsterile dosage forms.

Therefore, the current thinking on in-use stability testing by regulatory agencies and the guidelines for safe use proposed by health-care associations for sterile pharmaceutical dosage forms were examined in this study. In addition, the research trend related to the in-use stability of multiple-use sterile preparations for injections and ophthalmic solutions used for multiple use were overviewed. Based on this, detailed considerations such as test design contents that can be referred to when performing actual in-use stability for aseptic products in the pharmaceutical industry are provided, along with guidelines that regulatory authorities and health-care professionals can consider.

International regulatory and guideline trends: international organizations

International Conference on Harmonisation

The guideline on the photostability testing of new drugs (International Conference on Harmonisation guideline Q1B [ICH Q1B]), published in 1996 (ICH, 1996), states that it is appropriate to conduct this testing to prove photostability during use for some products such as infusions or dermal creams. The extent of this testing is determined according to the directions for use, and it is recommended that applicants choose arbitrarily (ICH, 1996).

According to the ICH Q5C guideline entitled "Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products," biotechnological/biological products are particularly sensitive to environmental factors such as temperature changes, oxidation, light, ionic content, and shear. To ensure the maintenance of biological activity and avoid degradation, stringent conditions for their storage are usually necessary, as reported in the Q5C guidance. According to Sect. 6.5 (Contained/Closure), the applicant should demonstrate that the closure used with a multiple-dose vial is capable of withstanding the conditions of repeated insertions and withdrawals so that the product retains its full potency, purity, and quality for the maximum shelf life specified in the instructions-for-use on containers, packages, and/or package inserts. Such labeling should be in accordance with relevant national/regional requirements (ICH, 1995).

On the other hand, based on the "Guidance for Quality Assessors—Drug Product" by the International Pharmaceutical Regulators Programme (IPRP) Quality Working Group, multiple-dose ophthalmic products with no in-use shelf life are assumed to have an in-use shelf life of 28 days. According to section P.8.1 (Stability Summary and Conclusion), data should be provided to support this period or a period that would cover the use of the entire product (IPRP, 2020).

International regulatory and guideline trends: regulatory authorities

Food and drug administration

According to the guidance for industry entitled "Selection of the Appropriate Package Type Terms and Recommendations

for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use," a multiple-dose container is a container of a sterile medication for parenteral administration (injection or infusion) that has met antimicrobial effectiveness testing requirements, or is excluded from such testing requirements by United States Food and Drug Administration (FDA) regulation (FDA, 2018).

According to the guidance for industry entitled "Current Good Manufacturing Practice—Guidance for Human Drug Compounding Outsourcing Facilities Under Section 503B of the Food, Drug, and Cosmetic Act," for multiple-dose products, appropriate laboratory tests and specifications include those for antimicrobial effectiveness, and whether the product contains a preservative or antimicrobial activity inherent in the formulation. If the acceptance criteria described in United States Pharmacopeia (USP) General Chapter < 51 > are met, labeling up to a 28-day in-use shelf life is considered appropriate for multiple-dose products (FDA, 2020).

USP convention

Based on USP43/NF38 General Chapter < 797 > Pharmaceutical Compounding—Sterile Preparations, three contamination levels for compounded sterile preparations (CSPs) were categorized (Table 1) into low-, medium-, and high-risk level CSPs (USPC, 2020).

The beyond-use date (BUD) after initially entering or opening (e.g., needle puncturing) multiple-dose containers is 28 days unless otherwise specified by the manufacturer. The majority of CSPs are aqueous solutions in which the hydrolysis of dissolved ingredients is the most common chemical degradation reaction. Drug hydrolysis rates increase exponentially with arithmetic temperature increase. BUDs for CSPs that are prepared strictly in accordance with manufacturers' product labeling shall be those specified in that labeling or from appropriate literature sources or direct testing. BUDs for CSPs that lack justification from either appropriate literature sources or by direct testing evidence shall be assigned as described in the "Stability Criteria and Beyond-Use Dating Under Pharmaceutical Compounding—Nonsterile Preparations" <795> (USPC, 2022).

Health Canada

According to the "Quality (Chemistry and Manufacturing) Guidance: New Drug Submissions (NDSs) and Abbreviated NDSs" by Health Canada, changes to a product after opening should be assessed for multiple-dose sterile products and for products where the labeling indicates a specific in-use shelf life. In-use shelf life should be justified with data where applicable and consistent with product labeling (e.g., in-use shelf life for ophthalmic products containing antimicrobial preservative should be justified with experimental data). Multiple-dose ophthalmic products with no in-use shelf life are assumed to have an in-use shelf life of 28 days. Data should be provided to support this period or a period that would cover the use of the entire product (Sect. 3.2.P.8.1 Stability Summary and Conclusion) (Health Canada 2018).

European Medicines Agency

According to the "Note for Guidance on Development Pharmaceutics by the European Medicines Agency (EMA)," in-use shelf life should be as short as possible, especially for large packs intended to be sterile, such as parenteral or ophthalmic preparations (Sect. 3.3 Liquid and Semi-Solid Formulations) (EMA, 1998). Based on the "Note for Guidance on Maximum Shelf-Life For Sterile Products for Human Use After First Opening or Following Reconstitution" by the EMA, the product should be used immediately from a microbiological point of view. If not used immediately, unpreserved sterile products should not be stored longer than 24 h at 2 °C to 8 °C, and aqueous preserved sterile products (including antimicrobial preservatives or intrinsically self-preserving) and nonaqueous sterile products (e.g., oily preparations) should not be stored longer than 28 days (EMA, 1998).

Pharmaceuticals and Medical Devices Agency

Based on the post-approval change review of the cartridge for insulin glargine injection (genetical recombination) by the Pharmaceuticals and Medical Devices Agency (PMDA), an in-use stability study was conducted at 30 °C for 32 days. It was confirmed that the in-use

Table 1 The shelf-life of compounded sterile preparations according to the risk level of microbial contamination without passing the sterility test [General Chapter < 797 >]

Classification Risk level of contamination	The shelf-life of compounded sterile preparations without passing the sterility test		
	controlled room temperature	refrigerated temperature	Freezing temperature between − 25° and − 10° (lyophilized solid state)
Low level	not more than 48 h	not more than 14 days	not more than 45 days
Medium level	not more than 30 h	not more than 9 days	not more than 45 days
High level	not more than 24 h	not more than 3 days	not more than 45 days

stability of the drug product during the in-use shelf life specified in the package insert was accurate because no substantial increase in high molecular weight proteins (HMWPs) was observed throughout the study period. Therefore, an in-use period of 28 days was proposed in Sect. 2.B.(2) (the effect of the change of plunger on product stability) (PMDA, 2014).

Based on the results of the review of the carteolol hydrochloride/latanoprost combination ophthalmic solution by the PMDA, the PMDA asked the applicant to explain any quality changes that might result during repeated use after opening the container. Assuming repeated use of a bottle of the drug product once daily for 1 month, the stability of the product (filled in a titanium oxide-containing polyethylene [PE] container) was studied by instilling a drop once daily and storing the container at uncontrolled room temperature and humidity under white fluorescence lighting (illuminance: 800 lx) for 35 days. No marked change was observed in the description, osmotic pressure ratio, pH, related substances by high-performance liquid chromatography (HPLC), foreign insoluble matter, and content (carteolol hydrochloride by HPLC and latanoprost by HPLC). Based on the study results, the PMDA accepted that this product should not be used approximately 1 month or later after opening the container (PMDA, 2016). The guidelines of international regulatory authorities are summarized in Table 2.

International regulatory and guideline trends: health-care associations

American Society of Health-System Pharmacists

Based on the "American Society of Health-System Pharmacists (ASHP) Guidelines on Compounding Sterile Preparations," the BUD is the date or time after which administration of a CSP shall not be initiated. The BUD is determined from the date or time the preparation is compounded, its chemical stability, and the sterility limits described later in these guidelines (ASHP guideline, USP Chapter < 797>). Both the stability of the components and the sterility limits described above must be taken into consideration when determining BUDs, and the BUD must be the shorter of the sterility dating or chemical stability dating in the expiration and BUD (ASHP, 2014).

Multiple-dose vials may be reused or saved up to the manufacturer's recommended BUD, if they are not opened in a direct patient-care area and if the facility's policy does not require a shorter period. If there is no manufacturer recommendation, multiple-dose vials may be reused or saved up to a maximum of 28 days or for a shorter period dictated by the facility's policy in the "Ampuls, Single-Dose, and Multiple-Dose Containers" section in the same guideline (ASHP, 2014). The person who first punctures a multiple-dose container intended for re-use must note the BUD and other information required by the facility's policy (e.g., his or her initials) on the vial or attached label. A label indicating "use by"

Table 2 Summary of the guidelines of international regulatory authorities

Organization	Target	Requirements
ICH	Finished drug products used after reconstitution or dilution	Stability after reconstitution or dilution
	Infusion or topical cream	photostability
	Multi-dose biological/biotechnological agents	Stringent conditions for their storage are usually necessary.
	multiple-dose ophthalmic products	Assumed to have an in-use period of 28 days.
FDA	multiple-dose products	Appropriate laboratory tests and specifications include ones for antimicrobial effectiveness, whether the product contains a preservative or antimicrobial activity is inherent in the formulation.
	multiple-dose products	Labeling up to a 28-day in-use shelf-life is considered to be appropriate.
USPC	multiple-dose containers otherwise specified by the manufacturer	The beyond-use date (BUD) after initially entering or opening (e.g., needle puncturing) multiple-dose containers is 28 days.
Health Canada	multiple-dose sterile products	The labelling indicates a specific in-use period.
	multiple-dose ophthalmic products with no in-use period	Assumed to have an in-use period of 28 days.
EMA	large packs intended to be sterile such as parenteral or ophthalmic preparations	In-use shelf life should be as short as possible.
	shelf-life for sterile products for human use after first opening or following reconstitution	The product should be used immediately from a microbiological point of view.
PMDA	multiple-doses insulin cartridge for injection	No substantial increase in high molecular weight proteins was observed
	combination Ophthalmic Solution	Shelf-life based on description, osmotic pressure ratio, pH, related substances by HPLC, foreign insoluble matter, and assay

clarifies that the date is the BUD rather than the opening date. If a vial lacks a BUD, it should not be used and should be properly discarded. The risk levels and BUDs of CSPs are presented in the "Risk Level Classification" section in the same guideline (ASHP, 2014).

National Association of Pharmacy Authorities

According to subsection 6.1 of the "Model Standards for Pharmacy Compounding of Non-Hazardous Sterile Preparations" and "Model Standards for Pharmacy Compounding of Hazardous Sterile Preparations" by the National Association of Pharmacy Regulatory Authorities (NAPRA), multiple-dose containers (e.g., vials) usually contain a preservative and the BUD is 28 days, unless otherwise specified by the manufacturer. If there is visible contamination before 28 days (or the manufacturer's expiry date), the container must be discarded (NAPRA, 2015; NAPRA, 2016). The BUD is based on the risk that a preparation may be contaminated according to USP/NF General Chap. 797.

National Health Service

In-use shelf life for eye preparations is suggested depending on the presence or absence of preservatives, based on the "Guidance on In-Use Shelf Life for Eye Drops and Ointments" by the National Health Service (NHS) Pharmaceutical Quality Assurance Committee and UK Ophthalmic Pharmacist's Group. In particular, for eye preparations containing preservatives, the in-use shelf life is set at 28 days. However, for high-risk eye preparations containing preservatives, the in-use shelf life is set at single use only for 7 days (NHS 2019). It is recommended that eye drops be discarded 1 month after opening unless otherwise stated by the manufacturer. It is recommended that insulin be stored in the refrigerator when unopened, and once in use, it should be kept at room temperature (25°) for 1 month (NHS 2021).

French Society of Clinical Pharmacy

According to the "Methodological Guidelines for Stability Studies of Hospital Pharmaceutical Preparations Part 1: Liquid Preparations" by the French Society of Clinical Pharmacy (SFPC) and Evaluation and Research Group on Protection in Controlled Atmosphere, the duration of microbiological stability for preparations produced in the hospital in accordance with the USP under reserve of physicochemical stability data was presented (SFPC, 2013).

Research trends on the in-use stability of sterile pharmaceutical dosage forms after opening

The in-use stability of insulin products among sterile biotechnological/biological products is summarized in Table 3.

Insulin lispro

The chemical and physical stability of the rapid-acting insulin analog, insulin lispro, was examined. Insulin lispro vials (100 U/10 mL) were administered as basal and bolus doses that simulate a patient's insulin pump and infusion system over a 14-day period under stressed conditions. The purpose of this study was to determine if aggregation or precipitation of the formulation due to these stresses may increase the risk of occlusion of the infusion set through the formation of fibrils or precipitates. There were two study arms: one with infusion set changes every 3 days and one with no infusion set changes throughout the study. The device used for evaluation was the MiniMed Paradigm Insulin Pump along with MiniMed plastic reservoirs and infusion sets. This simulated patient in-use study used basal and bolus doses of insulin lispro under conditions of elevated temperature at 37 °C and continuous shaking over 14 days. Samples were collected at the end of each bolus infusion day (Days 1, 5, 10, and 14). Analyses included physical appearance, pH, potency (U/mL), purity, HMWP content, and preservative (*m*-cresol) content. These analyses were measured with HPLC methods that followed those described in the USP monograph for insulin lispro injection (Sharrow et al., 2012). The pH of insulin lispro only slightly increased and remained well within the acceptance criterion of 7.0–7.8 for both arms. The antimicrobial agent m-cresol is present in insulin lispro vials and cartridges at a level of 3.15 mg/mL. Concentrations of m-cresol for samples from both study arms decreased over time but were well above the qualification limit of 1.15 mg/mL at all time points, which provides protection against microbial contamination. Both study arms showed an increase in the percentage of HMWP at all study time points compared with the baseline control sample, but remained below the 1.50% acceptance criterion. This study presented comprehensive stability-indicating data over an extended 14-day study period under conditions simulating patient use.

Insulin aspart, human insulin (recombinant DNA), insulin glargine, and insulin lispro

Heat stability studies of various insulin types in tropical temperature conditions were carried out using four analog insulin formulations (Kaufmann et al. 2021). According to

Table 3 Summary of in-use stability on multiple-use parenteral products (insulin products)

Product	Item	Summary
Insulin Lispro (Sharrow et al., 2012)	Container closure system	Medtronic Minimed paradigm® pump system
	Storage condition	high temperature (37 °C)
	Test interval	0, 1, 5, 10, 14 days
	Withdrawal method	
	Test measurements	physical appearance, pH, potency (U/mL), purity, high-molecular-weight protein (HMWP) content, preservative(m-cresol) content
	Stability	14 days
Insulin aspart Human insulin (rDNA) Insulin glargine Insulin lispro (Kaufmann et al. 2021)	Container closure system	vial or cartridge
	Storage condition	(1) fluctuation of tropical temperature conditions (25˚C ~ 37˚C) at 12 h-interval; (2) tropical temperature without fluctuation of temperature; (3) constant temperature conditions (31˚C or 37˚C)
	Test interval	(1) 0, 4, 12 weeks; (2) 0, 4 weeks; (3) 0, 4, 8 weeks
	Withdrawal method	insulin was taken out daily to simulate a typical daily treatment with dosages of 10 IU in the morning and 12 IU in the evening. (daily total 220 µL withdrawal (10 µL/IU)
	Test measurements	potency, biophysical assessment of secondary structure of insulin monomers, quantification of the preservatives, residual bioactivity measuring insulin efficiency on insulin receptor and Akt phosphorylation in two different hepatocytes cell lines
	Stability	4 weeks
Human insulin (recombinant DNA) (Erdnüß et al. 2022)	Container closure system	(1) 50 mL syringe (test solution); (2) infusion bag (bulk solution)
	Storage condition	(1) 2–8 °C/protected from light; (2) 20 ~ 25 °C/diffuse room light 3) for 28 days/ protected from light at 2–8 °C and additional 24 h at 20–25 °C/diffuse room light
	Test interval	(1) 0, 7, 14, 21, 28, 58, 90 days; (2) 0, 4, 8, 12, 24 h, 2, 7, 14, 21, 28, 58, 90 days 3) 0, 4, 8, 12, 24, 48 h, 5 days
	Withdrawal method	
	Test measurements	determination of human insulin (reversed-phase HPLC), pH (only storage condition 3), visible/subvisible particles (only storage condition 3)
	Stability	90 days when stored at 2–8 °C/ protected from light in 50 mL syringes
Insulin (Kongmalai et al., 2018)	Container closure system	cartridge
	Storage condition	room temperature, refrigerated temperature, the worst temperature condition protected from light (37 °C)
	Test interval	0, 28 days
	Withdrawal method	all of the insulin pens were opened and flipped back and forth for 20 times and two units of insulin were discarded every day
	Test measurements	insulin quantity
	Stability	28 days for all of the storage conditions

pharmacopeia, unopened insulin vials must be stored in a refrigerator (2–8 °C), while storage at ambient temperature (25–30 °C) is usually permitted for a 4-week usage period during treatment. According to manufacturers' specifications, the maximum ambient temperature should not exceed 25 to 30 °C during this period; however, in tropical regions, ambient temperatures often exceed these limit values. Therefore, the question is whether commercially available insulin is stable under high oscillating ambient temperatures for a period of 4 weeks (28 days), which corresponds with the usual recommended period of use of most commercially available insulin upon opening of the vial or cartridge. In this study, researchers analyzed the quality of insulin formulations stored under different conditions with respect to structure and efficacy. First, temperature cycles were programmed as follows: start at 25 °C, increase to 37 °C in 12 h, and then decrease back to 25 °C in 12 h, in a continuous loop mode. Second, samples including vials or cartridges of each tested formulation were submitted to temperature cycles, and insulin was taken out daily to simulate a typical daily treatment with dosages of 10 IU in the morning and 12 IU in the evening. Third, since the median temperature during the cycling process remains above 31 °C as indicated by the manufacturers, a series of vials and cartridges were exposed continuously to this temperature, and insulin quantification was performed after 1, 4, and 8 weeks of this treatment. Additionally, the two insulin formulations considered essential medicines by the World Health Organization (rapid and neutral protamine Hagedorn [NPH]/isophane insulin) were exposed to a continuous temperature of 37 °C. Three parameters were investigated in the insulin formulations (rapid, NPH/isophane and mixed insulin): (i)

potency determination by HPLC, which is the gold standard method for insulin stability assessment according to major pharmacopeias; (ii) biophysical assessment of the secondary structure of insulin monomers by circular dichroism; and (iii) residual bioactivity measuring insulin efficiency on the insulin receptor and Akt phosphorylation in two different hepatocyte cell lines (Kaufmann et al. 2021). A complete series of vials or cartridges of the insulin formulations were stored at 4 °C and used as references for all three analytical methods. All insulin formulations started to degrade after 4 weeks (degradation proportion between 5% and 13.5%, compared with values obtained for the corresponding formulations at T = 0). There was further degradation after 8 weeks (12–19%), with all formulations out of range accepted by the pharmacopeia (100 ± 10%) in the third group. These findings showing that insulin retains structural and efficacy integrity over a period of 4 weeks' exposure to oscillating temperatures, open new perspectives for diabetes care in regions where strict storage at 2–8 °C is not available at the patient level.

Human insulin (recombinant DNA)

The objective of this study was to investigate the physicochemical stability of ready-to-administer human insulin (HI) at 1 IU/mL injection solutions of two different brand products diluted with 0.9% sodium chloride solution under different storage conditions, either in 50 mL disposable plastic syringes or as bulk solution in infusion bags. EMA-approved HI injection solution at 100 IU/mL produced by recombinant DNA technology was used (Erdnüß et al. 2022). Three test solutions of each HI brand product prepared in the different types of 50 mL plastic syringes were stored for 90 days, either at 2–8 °C/dark or at 20–25 °C/diffuse room light. Ten test solutions of each HI brand product prepared in the different types of 50 mL plastic syringes were stored for 28 days/dark at 2–8 °C and an additional 24 h at 20–25 °C/diffuse room light. Bulk solutions in 3-L infusion bags (one EVA and one polyolefin infusion bag) were stored for 5 days at 2–8 °C/dark. Samples were taken on Days 0, 7, 14, 21, 28, 58, and 90 for test solutions stored at 2–8 °C. Sampling was performed at 0, 4, 8, 12, and 24 h and on Days 2, 7, 14, 21, 28, 58, and 90 for test solutions stored at 20–25 °C in plastic syringes. Sampling was taken at 0, 4, 8, 12, 24, and 48 h and on Day 5 for test solutions stored in the dark at 2–8 °C and an additional 24 h at 20–25 °C in diffuse room light. For the determination of HI concentrations, we used a stability-indicating reverse-phase HPLC assay. The 50 mL syringes remained physiochemically stable for up to 90 days when stored at 2–8 °C/dark and for at least 14 days when kept at 20–25 °C/diffuse room light. For the preparation of bulk solutions, the prefilled polyolefin infusion bag was appropriate.

Insulin

Four different commercial basal insulins—namely, long-acting insulin, NPH-1, NPH-2, and NPH-3—were evaluated because most basal insulin was used once daily. Furthermore, the majority of patients keep their insulin pen at room temperature at home, which is likely to be exposed to high temperatures after opening (Kongmalai et al., 2018). Manufacturers recommend that used inulin pens be maintained at room temperatures not exceeding 25–30 °C because purified insulin is degraded by deamidation and polymerization, but room temperature in tropical countries is normally higher. The primary objective of this study was to compare the stability of opened insulin in three different storage conditions (room temperature, refrigerator, and incubator [37 °C]) with unopened insulin in the refrigerator. In the experimental groups, all of the insulin pens were opened and flipped back and forth 20 times. Two units of insulin were discarded every day to simulate the daily usage of insulin-treated patients with diabetes. The estimated sample size was five samples of each insulin type for each of three storage conditions and a control group. There are four insulin types, so the total sample size was 80. There were no statistically significant differences in insulin quantity during the 28 days the samples were stored in the refrigerator, room temperature, or incubator.

Research trends on the in-use stability of ophthalmic solutions

The in-use stabilities of ophthalmic solutions are summarized in Table 4.

Atropine

The aim of this study was to assess the physicochemical stability and control the sterility of two 0.1 mg/mL atropine ophthalmic solutions (with and without an antimicrobial conservative) in two different low-density PE (LDPE) multidose eyedroppers (one with a sterility-preserving technology allowing the absence of an antimicrobial preservative in the formulation and the other without such a system to allow the choice of container depending on the stability data) at 25 °C for 6 months in unopened eyedroppers (Berton et al. 2020). Two different formulations of 0.1 mg/mL (0.01%) atropine ophthalmic solutions were prepared: one was atropine solution with an antimicrobial preservative (cetrimide) for use with ethylene oxide sterilized white

Table 4 Summary of in-use stability on ophthalmic preparations

Product	Item	Summary
Atropine ophthalmic solution (0.1 mg/mL) (Berton et al. 2020)	Container closure system/Preservatives	1)white opaque low-density polyethylene (LDPE); 2) gamma-sterilized white opaque LDPE squeezable multidose eyedropper equipped with sterility preserving Novelia® caps/1) 0.01% cetrimide; 2) No
	Test interval	0, 1, 2, 3, 4, 5, 6 days
	Withdrawal method	one drop from each eye droppers was manually emitted at room temperature each day
	Test measurements	Atropine quantification
	Stability	6 days
Chlorhexidine digluconate (Lin et al. 2015)	Container closure system/Preservatives	light-resistant high-density polyethylene (HDPE)/No
	Test interval	0, 1, 2, 3, 4, 5 weeks
	Withdrawal method	In the daily-open groups, authors opened the eyedroppers thrice daily (at 9:00 AM, 1:00 PM, and 5:00 PM), shook them for a while, and let the eyedroppers stand for 10 min prior to being closed.
	Test measurements	assay, pH, particulate matter, osmolality, sterility (USP)
	Stability	stable for 1 month
Cyclosporine A (Chennell et al. 2017)	Container closure system/Preservatives	a low density polyethylene multidose eyedropper and sterility preserving cap (Novelia®)/No
	Test interval	0, 1, 7, 30 days
	Withdrawal method	each day, 1 drop of the contained solution of cyclosporine A was pressed out through the Novelia® tip.
	Test measurements	visual inspection, cyclosporine quantification, osmolality, and pH measurements. Turbidity, sterility
	Stability	30 days
Polyhexamethylene biguanide (2 mg/mL) (Bouattour et al. 2018)	Container closure system/Preservatives	(1) gamma-sterilized white opaque low-density polyethylene (LDPE) and sterility preserving Novelia® caps; (2) gas-sterilized white opaque low-density polyethylene/No
	Test interval	0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 days
	Withdrawal method	on the drops emitted from the multidose eyedroppers to simulate in-use condition
	Test measurements	Quantification of polyhexamethylene biguanide
	Stability	10 days
Tacrolimus (0.2 mg/mL and 1 mg/mL) (Bouattour et al. 2018)	Container closure system/Preservatives	low-density polyethylene bottle and sterility preserving Novelia® nozzle/No
	Test interval	28 days (0, 24, 33, 48, 57, 72, 81, 96, 105, 168, 177, 240, 249, 336, 345, 432, 441, 672, 681 h)
	Withdrawal method	opened and one drop from each eyedropper was manually emitted out of the bottle and collected for analysis each day (except weekends) twice a day (morning and evening) for one month and tacrolimus concentration was measured in the emitted drops.
	Test measurements	pH, osmolality, tacrolimus quantification
	Stability	28 days

opaque LDPE squeezable multidose eyedroppers (reference VPLA25B10; Laboratoire CAT®, Lorris, France); the other was atropine solution without preservative for use within a gamma-sterilized white opaque LDPE squeezable multidose eyedropper (reference 10,002,134) equipped with sterility-preserving Novelia® caps (reference 20,050,772; Nemera, La Verpillère, Cedex France). In an in-use study, atropine quantification was also performed on the drops emitted from the multidose eyedroppers. For the evaluation of atropine concentrations in eye drops during simulated use, 30 eyedroppers were subjected to simulated patient use: every day for 6 days, one drop from each eye droppers was manually emitted (i.e., the drop was squeezed out of the bottle as if to be administered to the eye, but instead of being administered, it was collected for analysis) at room temperature. Then atropine quantification was conducted in triplicate from 10 collected and pooled drops. In between use, the bottles were stored vertically at 25 °C. During the 6-day study, atropine concentrations in the emitted drops remained stable (no variation exceeding ±5% of the initial concentration), thereby excluding any clinically significant loss of atropine. Such information is of high clinical value. It also indicates that the preservative-free formulation can be used safely with the sterility-preserving device that was tested (using Novelia® caps).

Chlorhexidine digluconate

The aim of this study was to develop an optimal extemporaneous 0.02% chlorhexidine digluconate ophthalmic formulation with an appropriate pH value and osmolality for patients with *Acanthamoeba* keratitis, in order to ensure that the sterility, particulate matter, and assay of the final product meet the general characteristics of ophthalmic solutions in pharmacopoeia, and to determine the type of container, storage condition, and BUD of the final product. The experimental design and sample preparation assessed were solvent, type of container, storage temperature, opened three times daily/sealed, and test time points. Stability parameters such as assay, pH value, osmolality, particulate matter

concentration, and sterility were also assessed. The authors simulated the contact time of the solution to the air in clinical settings by study design. In the daily-open groups, the authors opened the eyedroppers three times daily (9:00 AM, 1:00 PM, and 5:00 PM), shook them for a while, and let the eyedroppers stand for 10 min prior to being closed. When stored at 2–25 °C, the formulation has a 6-month BUD when sealed in a light-resistant high-density PE dropper, and is stable for 1 month when opened for 10 min three times daily.

Cyclosporine A

Authors investigated the physicochemical and microbiological stability of two high concentrations (10 and 20 mg/mL) of an ophthalmic cyclosporine A micellar solution in a low-density PE multidose eyedropper at two conservation conditions (5 and 25 °C), before and with simulated use (Chennell et al. 2017). The stability of the 10 and 20 mg/mL cyclosporine A micellar solutions was studied in unopened multidose eyedroppers for 6 months at 25 °C or 5 °C, and in simulated use conditions, for 1 month. On Day 30, 16 eyedroppers per tested concentration and storage temperature were subjected to simulated normal patient use; each day, one drop of the contained solution of cyclosporine A was pressed out through the Novelia® tip. The following analyses were performed after 1, 7, and 30 days of simulated use: visual inspection, cyclosporine quantification, osmolality, and pH measurements. Turbidity measurements were performed on the pooled volume from the four units at the previously stated times (after 1, 7, and 30 days of simulated use) for the 10 mg/mL formulation (turbidity measurements were not performed for the 20 mg/mL formulation due to high variability in measurements linked to microbubbles caused by volume pooling). Cyclosporine quantification was also performed on four pooled drops emitted per concentration and storage condition. A sterility assay was performed on Day 30 of simulated use on the four units. Furthermore, for each concentration, the weight of five drops emitted from three eyedroppers (15 drops in total) was measured, and the mean drop volume was calculated using the solution's known density. The micellar formulation presented in this study can therefore be stored at 5 °C or at ≤25 °C for up to 6 months.

Polyhexamethylene biguanide

The objective of this study was to assess the physicochemical stability and control the sterility of a polyhexamethylene biguanide (PHMB) 0.2 mg/mL (0.02%) ophthalmic solution in two different low-density PE multidose eyedroppers, at two conservation conditions (5 and 25 °C) for 3 months in unopened eyedroppers and during 10 h of simulated patient use (Bouattour et al. 2018). PHMB quantification and breakdown product screening were also performed on the drops emitted from the multidose eyedroppers to simulate in-use conditions. On Day 0, 10 eyedroppers for each tested condition were subjected to simulated patient use: every hour for 10 h, a drop was emitted, and PHMB quantification was realized in 10 pooled drops of the 10 bottles per conditioning and per storage temperature condition.

Tacrolimus

The objective of this study was to develop a physicochemically stable, preservative-free formulation of tacrolimus, at two different concentrations—0.2 mg/mL (0.02% m/v) and 1 mg/mL (0.1% m/v)—to cover a wide therapeutic spectrum of ophthalmic illnesses (vernal keratoconjunctivitis and allergic eye diseases) (Barrieu et al. 2022). The stability of the tacrolimus formulations was studied in unopened eyedroppers stored in the dark vertically for 9 months at different storage temperatures: 5 ± 3 °C (Liebherr refrigerator), 25 ± 1 °C and 35 ± 1 °C with 60 ± 5% residual humidity in a validated climate chamber (Binder GmbH, Tuttlingen, Germany). A simulated use study was also performed during the 28 days at 5 °C after 8 months of storage at 5 °C for both concentrations. In the in-use study, tacrolimus quantification was also performed on the drops emitted from the eyedroppers. A simulation of patient use was performed after 8 months of storage at 5 °C. Eight units of each concentration were opened, and one drop from each eyedropper was manually emitted out of the bottle and collected for analysis every day (except weekends) twice a day (morning and evening) for 1 month and tacrolimus concentration was measured in the emitted drops. To ensure that a sufficient volume was collected to allow tacrolimus quantification, at each analysis time, the drops were pooled by two (each time from the same two vials, which remained associated throughout the analysis). Thus, the results of these analyses were returned with n = 4. The units were stored vertically at 5 ± 2 °C between drop collection. After the 28 days, the units were opened and the remaining solution was subjected to tacrolimus quantification as well as pH and osmolality measurements. Tacrolimus ophthalmic micellar solutions at 0.2 and 1 mg/mL are physiochemically stable for up to 9 months when stored at 5 °C, but additional studies are needed to evaluate the in-depth container–content interactions that were detected, especially the impact of a compound leaching out of the used eyedropper bottle.

Considerations for the in-use stability of sterile preparations

First, as mentioned in the guidelines of regulatory authorities and health-care associations (FDA, 2020; ASHP, 2014), it is necessary to consider sterility as well as the stability of the component itself when setting the in-use shelf life due to the nature of sterile preparations. It is considered necessary to check the appropriate antimicrobial efficacy of the product when setting the in-use shelf life, depending on whether the formulation contains an antimicrobial preservative or whether the active ingredient itself contains intrinsic antimicrobial efficacy.

Second, regarding the storage conditions of the in-use stability test, it should be stored under the permitted storage conditions during the test period, as well as in harsh conditions (e.g., tropical climate conditions) as in the case of the abovementioned insulin product after opening, as there may be cases where the clinical use environment is required (Sharrow et al., 2012; Kongmalai et al., 2018; Kaufmann et al. 2021). These harsh conditions (e.g., high temperature) were used for stability testing under storage conditions after opening. It is necessary to include the worst conditions (e.g., high temperature) as well as general storage conditions, or as intermediate test conditions or accelerated test conditions specified in the ICH Q1A stability test guidelines (ICH, 2003).

Third, regarding the test period (cycle) and in-use simulation design (withdrawal method), it is necessary to reflect and present the actual clinical use environment, such as dosage, in detail. In particular, in the case of eye drops, the number of administration times (e.g., administration of a few drops several times a day) and duration of administration, as well as the worst case where repetitive exposure to the external environment is expected to be highest when opening and closing the container closure system (e.g., applied to both eyes, the larger of several packaging units), can be applied including withdrawal methods. The in-use stability test period can be set (for at least 28 days) considering the period in which the packaging unit is expected to be completely consumed according to application of the withdrawal method.

Fourth, according to the aforementioned in-use stability studies (Sharrow et al., 2012; Ratprasatporn et al., 2019; Kaufmann et al. 2021) and the study by Choi et al. (2022), if possible, in addition to the test group (opened group), negative control group (unopened), and positive control group (remained open throughout the test period), applying the worst environment (e.g., temperature, humidity, light) is also considered desirable.

Fifth, the test method and test measurements are expected to change after opening to properly evaluate the physicochemical and microbiological properties of the product according to the guidelines of the relevant regulatory authorities (EMA, 2001; MFDS, 2016). The method can be set at least the appearance, pH, assay or potency, purity, if necessary, the content of preservatives (or preservative efficacy testing) in the case of insulin preparations. It may include pH, assay or potency, purity, osmotic pressure, insoluble matter, and, if necessary, preservative content (or preservative efficacy testing) in the case of eye drops. In particular, in the case of sterile preparations, to ensure the sterility of these products, it may be necessary to set the sterility test or microbial limits taking into account the in-use condition of the products.

Sixth, in the case of eye drops, since the packaging unit is generally small compared with other formulations, it will be possible to test by pooling several packaging units to secure the sufficient volume of test samples, and pooling in the same manufacturing unit as much as possible for the uniformity of test results is recommended.

Seventh, in general, it is recommended that aseptic preparations without preservatives be used immediately after opening in the regulatory guideline discussed above (EMA, 1998). However, in the case of eye drop formulations, special container closure systems such as sterilized closure have been developed recently and there are cases where the in-use shelf life is set based on the results of the in-use stability test (Chennell et al. 2017; Bouattour et al. 2018; Berton et al. 2020; Barrieu et al. 2022). In the case of preservative-free eye drops using a brand-new container closure system to guarantee the sterility, it is considered that the in-use stability should also be considered, as in the case of multiple-use eye drops containing preservatives.

Last, in the case of insulin preparations, considering the absorption of the corresponding relevant ingredient to the infusion system, it is necessary to separately consider the in-use stability of the system. In this case, it is considered that stability testing (e.g., withdrawal method) be applied after opening in a similar way to eye drops.

Apart from this, in relation to the clinical use environment, it is thought that health-care workers can refer to the in-use period according to the risk of microbial contamination mentioned in the guidelines of the relevant regulatory authorities and health-care associations.

For other matters related to in-use stability, it is believed that the recommendations for in-use stability tests for biological products and nonsterile products can be referred to in the guidelines of the relevant regulatory authorities (EMA, 2001; MFDS, 2016; IPRP, 2020) and related review papers (Ricci et al. 2015; Choi et al. 2022; Lee et al. 2022). However, it may be necessary for the regulatory authority to present specific and subdivided considerations for each

formulation for a clearer test design and execution by the applicant.

Conclusion

In this study, we examined the guidelines of regulatory authorities and health-care associations related to the in-use stability testing of sterile preparations and related research trends of in-use stability testing. Based on these results, the storage conditions and tests related to the in-use stability testing of sterile preparations were reviewed. Specific considerations such as period, test intervals, in-use simulation design (withdrawal method), and test measurements were presented. Protection against contamination must be ensured for reconstituted medicines. The pharmaceutical industry has already sufficiently considered this issue, which is reflected in the approval requirements. Therefore, the literature on formulations expected to have stability issues after opening were examined in this review, with reference to the guidelines of related regulatory agencies and health-care and drug organizations.

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Declarations

Conflict of interest All authors (H.L. Yoo, Y.G. Na, M.K. Jin, J.H. Won, B.M. Song, T.S. Yun, Y.R. Hwang, H.K. Lee and C.W. Cho) declare that they have no conflict of interest.

Research involving human and animal rights This article does not contain any studies with human and animal subjects performed by any of the authors.

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