DETERMINATION OF PRESERVATION EFFICACY IN WATER-MISCIBLE PERSONAL CARE PRODUCTS

INTRODUCTION

Many personal care products provide conditions that are conducive to microbial growth such as water, nutrients, and favorable pH. Preservation efficacy testing (PET) is performed to assure that each personal care product that is susceptible to microbial growth is not affected by the introduction of microorganisms during normal or reasonably anticipated use by the consumer. However, some personal care products, due to characteristics that create an environment hostile to microbial growth and/or survival, are considered low microbiological risk[1] (see also Section 15 - Microbiological Risk Factor Assessment of Atypical Cosmetic Products and Reference #1). These products are not routinely subject to preservative efficacy testing.

The design of preservation tests and subsequent interpretation of results is a complex process. The technical personnel responsible for preservation testing should therefore, be professionally educated and experienced in conducting challenge test procedures and evaluating the data generated (see Sections 4 and 9 – Microbiology Staff Training and Microbial Validation and Documentation).

It is important to remember that microorganisms are ubiquitous and capable of adaptation. No method can guarantee adequate microbiological control under all conditions. In addition, the importance of adhering to good manufacturing practices in the production of personal care products cannot be overstated. Preservatives should not be used as a substitute for good manufacturing practices[2].

Personal care products constitute a wide and expanding variety of formulations and package configurations that differ significantly from each other in their composition, intended use, and physical characteristics. This guideline reflects current considerations and practices used within the personal care industry in conducting preservation efficacy testing of water-miscible products. Other standardized methods as well as those developed by the user may also be employed to assess preservation efficacy of water-miscible personal care products[3,4] .For guidance on testing atypical products, see Section 15 - Microbiological Risk Factor Assessment of Atypical Cosmetic Products.

SCOPE

The purpose of this guideline is to provide guidance to those developing and performing PET of water-miscible personal care products such as are described in Method M-3, A Method for Preservation Testing of Water-Miscible Personal Care Products (Section 19) and Method M-7, A Rapid Method for Preservation Testing of Water-Miscible Personal Care Products (Section 22).

GENERAL CONSIDERATIONS

The preservation efficacy test (PET) is an important tool in evaluating the robustness of a formulation to withstand microbial contamination during consumer use. Various factors can have an effect on the performance of a preservative system in a formulation and on the interpretation of results generated from these studies[1,5-7].

The following factors may be considered when performing a preservation efficacy test and interpreting results:

- The nature and compatibility of the raw materials in the formulation [6].
- Information on preservation of similar formulations.
- Manufacturing conditions, e.g. temperature of processing and filling [6].
- Types of packaging used to contain and deliver the product [1,5, 7].
- Information on the product's intended use, including area of application, frequency of use, shelf-life, intended users, etc.

PRODUCT TESTING

Microbial Content Test

It is recommended that the bioburden of the test sample(s) be determined by a microbial content test, such as Method M-1 - Determination of the Microbial Content of Cosmetic Products (Section 17), prior to performing the preservation efficacy test. The purpose is to verify that the level and type of microorganisms in the test sample will not interfere with the recovery of the test organisms or interfere with the interpretation of the challenge test data. Furthermore, an initially high microbial bioburden in the test sample before inoculation with microorganisms could compromise the preservation system.

Neutralization

Antimicrobial or preservative neutralizers are added to microbial plate count diluents and recovery agars to inhibit or inactivate the antimicrobial properties or activity of the formulas being tested[8]. Carryover of antimicrobial activity from the product formulation into the microbial plate count diluent and recovery growth agar may partially or completely inhibit the growth of surviving challenge test microorganisms[9]. Antimicrobial or preservative neutralization may normally be accomplished by use of chemical neutralizing agents, physical dilution, or a combination of both. Prior to

the start of the neutralization step of the test, verification of the lack of neutralizer toxicity to each of the challenge test microorganisms must be confirmed [10]. Once this has been confirmed, this step does not need to be repeated during routine neutralization studies of formulations using the same microbial count diluent or recovery agar. If a new preservative neutralizing agent is added to the microbial count diluent or recovery agar, verification of the lack of neutralizer toxicity to each of the challenge test microorganisms must be re-confirmed.

Examples of chemicals and agents that may be used to neutralize the antimicrobial activity of preservatives are listed in Table 13-1[11]. Verification of the antimicrobial or preservative neutralization is generally performed by inoculating the product dilution and a microbial count diluent without product (control) with a low level of challenge microorganisms to yield a final count of approximately 30 to 300 Colony-Forming Units (CFU)/milliliter (ml) for each challenge test microorganism in a product test dilution and diluent control. Enumeration of the microorganisms from these dilutions is performed. Neutralization of the antimicrobial or preservative activity is verified if microbial recoveries of both test and control dilutions are within 0.5 log of each other. If one or more challenge test microorganisms cannot be recovered, the use of a higher dilution and/or the investigation of additional chemical neutralizers may be considered.

TEST PROCEDURE

Organisms

The organisms listed in Table 19-1 of Method M-3 (Section 19) and Table 22-1of Method M-7 (Section 22) are representative types of the microbial species that a formulation may encounter during manufacture and use: Gram-positive cocci, Gram-negative fermentative bacilli, Gram-negative non-fermentative bacilli, yeast and

mold. It is recommended that at least one microorganism from each group be included in the challenge test. Examples of other organism types, relevant to the formulation, may include microbial isolates that have been recovered from raw materials, formulations returned from consumers or other sources (e.g. product in or after use studies). Either pure or mixed microbial culture suspensions may be used to conduct challenge testing of formulations. Decisions to use pure or mixed cultures may be influenced by the factors discussed below.

Inocula consisting of only pure microbial cultures will yield specific data on each test microorganism employed in the challenge study. Mixed culture inocula may serve to simulate real world conditions during use. When conducting mixed culture challenge studies, it is recommended that closely related types of microorganisms such as Gram-positive bacteria (e.g. cocci), Gram-negative fermentative bacilli, Gram-negative non-fermentative bacilli, and yeasts and molds be pooled into separate distinct groups. Antagonism between different types of organisms may occur due to differences in growth factors and nutritional requirements[12]. A rapidly growing organism may impede the detection of a more slowly growing organism. For example, *Escherichia coli* has a shorter generation time and may obscure detection or growth of microorganisms such as *Pseudomonas aeruginosa* that have a longer generation time. Competition for growth factors or production of inhibitory byproducts and other factors may result in antagonism between different types of microorganisms[12, 13].

Inoculation and Enumeration Procedures

A typical challenge test consists of the following steps: inoculation of the test formulation, followed by enumeration of the inoculated formulation at various time points, and interpretation of data to determine adequacy of preservation. Details of these procedures may be found in the challenge test methods of M-3 (Section 19)

and M-7 (Section 22). It is recommended that the volume of inoculum added to the sample of the formulation does not exceed 1% by volume. Larger volumes of inocula (e.g. >1.0%) may result in undesirable dilution of the test formulation.

A preservative challenge test usually employs a single inoculation of each microorganism or pool of microorganisms. A rechallenge consisting of a second inoculation may be considered if more information is desired, e.g. to determine if a formulation is marginally preserved.

OTHER CONSIDERATIONS

Modified Formulations

When changes are made to the composition of a formulation that has already been challenge tested, a rapid screening test such as described in Method M-7 (Section 22) may be used to determine whether the change has an adverse effect on the preservation adequacy of the formulation. The decision to perform additional challenge testing for these types of formulations is dependent upon, but not limited to the type of finished package used, pH changes to the formulation, the addition of new or deletion of raw ingredients from the previously tested formulation, and the challenge test data of the original tested formulation.

Scale-Up/Pilot Batches

Changes in processing conditions during scale-up from laboratory to production size batches may alter the performance of the preservation system. Processing conditions (e.g. order of raw ingredient addition, pH of a batch during processing, and temperature of a batch during processing) may alter the antimicrobial activity or the physical stability of the preservative system in a formulation. Therefore,

preservation tests may be performed on scale-up batches to confirm the effectiveness of the preservation system.

Product Stability

During product development, the stability of the preservation system in a formulation should be considered. Challenge tests may be performed on either bulk material or product from a filled container that has been aged by using accelerated aging conditions such as holding at specific temperature and/or humidity conditions or real time aging at ambient conditions. Accelerated aging may cause a formulation to undergo chemical and physical changes more rapidly than would otherwise occur during real time aging. A decrease in preservative effectiveness over a period of time may occur due to a variety of factors. These factors include preservative degradation, partitioning, interaction with other formula ingredients, and chemical reaction with or absorption into the packaging material. The PET may be used to assess the degree of preservative effectiveness after accelerated or real time aging of a formulation has occurred. The preservative challenge test criteria for accelerated or real time aged product may or may not differ from the challenge test criteria for fresh product.

RECOMMENDATIONS

Since many personal care products are used on a regular basis, an effective preservation system should ensure the reduction of bacteria to a low and steadily decreasing level and fungi should remain static or decrease over time, even after repeated use by the consumer. The following challenge test criteria are the minimal recommendations for evaluating preservation system performance in water- based product formulations:

- Vegetative Bacteria

  There should be greater than or equal to 3 log (≥99.9%) reduction of vegetative bacteria by aerobic plate count or quantitative spread plate methods within 7 days following each challenge and no increase to the end of the test period.

- Yeast and Molds

  There should be greater than or equal to 1 log (≥90%) reduction of yeasts and molds by aerobic plate count or quantitative spread plate methods within 7 days following each challenge and no increase for the duration of the test period.

- Spore-Forming Bacteria

  If spore-forming bacteria are included in the test, there should be bacteriostatic activity against these microorganisms throughout the entire test period.

The above minimal challenge test criteria are suggested to aid manufacturers in evaluating the adequacy of preservation in personal care products. If a product does not meet these criteria, it is the responsibility of the manufacturer to select the appropriate challenge test criteria that will ensure product integrity. For example, single use packaging or use of pressurized delivery systems may be factors that could be considered in selecting appropriate criteria. More stringent challenge test criteria may be considered where deemed appropriate.

Table 13-1 – Examples of Antimicrobial Preservatives and Recommended Neutralizing Agents

Antimicrobial Preservatives	Recommended Neutralizing Agents:
Alcohol	Dilution, Nonionic Surfactants (e.g. Polysorbates)
Bronopol (2-Bromo-2-Nitropropane-1,3,-Diol)	Sulfhydryl Compounds (e.g. Cysteine, Thioglycollate, Thiosulfate, and Metabisulfite)
Chlorhexidine	Nonionic Surfactants (e.g.Polysorbates) and Lecithin, Anionic Surfactants
Formaldehyde donors	Dilution, Protein, Gelatin, Sodium bisulfite, Histamine, Histidine, Nonionic Surfactants (e.g. Polysorbates), Lecithin
Glutaraldehyde	Dilution, Sodium bisulfite, Sodium sulfite, Sodium thioglycollate, Glycine
Isothiazolinones	Dilution, Amines, Sulfites, Sodium bisulfite, Sodium thioglycollate, Mercaptans
Organic Acid Preservatives (e.g. benzoic and sorbic acids)	Nonionic Surfactants (e.g. Polysorbates), increasing pH
Mineral Acids (e.g. sulfuric and hydrochloric acids)	Increasing pH, peptones
Parabens	Lecithin, Nonionic Surfactants (e.g.Polysorbates),
Phenolic Compounds (e.g. Phenylphenol, chloroxylenol, cresols, chlorocresols)	Nonionic Surfactants (e.g.Polysorbates) and Lecithin
Quaternary Ammonium Compounds	Lecithin, Nonionic Surfactants (e.g.Polysorbates), Protein, Anionic Surfactants

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