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Research paper

Extension of in-use stability of preservative-free nasalia

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Abstract

Nasal drops and nasal sprays are commonly supplied in multi-dose containers that usually include suitable levels of an appropriate preservative in order to kill or prevent growth of any microorganisms which might enter the dispensing system. Preservatives should both protect the patient from infection and prevent spoilage of the product. Unfortunately, preservatives often cause unwanted side effects; in particular, the nasal mucosa is irritated frequently. Consequently, the use of preservatives in nasal preparations should be avoided. The technical design of the 3K system, a new multi-dose container, combines several microbiological safety features and therefore allows use without preservatives. Earlier tests have shown its safety for 6 weeks after the first opening. In order to test the microbiological safety of this multi-dose system over longer time periods, an in-use stability test was designed. The results revealed that the first dose as well as the contents complied with the requirements of the European Pharmacopoeia. Therefore, from a microbiological point of view for the tested nasalia in the 3K system, the stability after opening could be extended from 6 weeks up to several months without loss of microbiological quality.

Keywords: Nasalia; In-use stability; Preservative-free; Multi-dose container; 3K system; Drug delivery; Microbiological safety

1. Introduction

The European Pharmacopoeia (Ph. Eur.) specifies that the total viable aerobic count of nasal preparations should not exceed 10^2 colony forming units (CFU) per ml. Usually this goal is accomplished by including suitable levels of an appropriate preservative in the preparation in order to ensure that any microorganisms are killed or prevented from growing. The effectiveness of preservatives is based on biocidal and killing properties against most germs. Nevertheless, most preservatives show undesirable side effects that influence the clearance and defense mechanisms of the nose such as inhibition of cilia motility [1–3]. Scientists as well as physicians are concerned about the use of preservatives and their side effects and therefore recommend avoiding preservatives in nasal formulations whenever possible [4]. Preservative-free preparations must however comply with the above-mentioned requirement of Ph. Eur. in the same way as conventional preservative-containing preparations.

During normal use of a nasal spray in a multi-dose container, there are two main microbiological risk factors: (a) the external orifice where microorganisms are deposited, leading to the formation of a liquid film through which entry into the container might be gained and (b) the potentially contaminated air which enters the system when the pump is used. Therefore, it is essential to ensure that the numbers of any microorganisms which might penetrate the semi-exposed zone below the orifice and thus contaminate the next dose are effectively reduced and that the container is adequately protected from the external environment so that no microorganisms can enter the main reservoir. In the 3K system, the aim of a multi-dose container for drug delivery of preservative-free products such as nasalia is achieved via the combination of several safety features that generate a multiple protected system (Fig. 1): silver (silver coil, No. 3), which has an oligodynamic (antimicrobial) effect [5], is incorporated in the system in order to reduce the numbers of any microorganisms which penetrate the dead space

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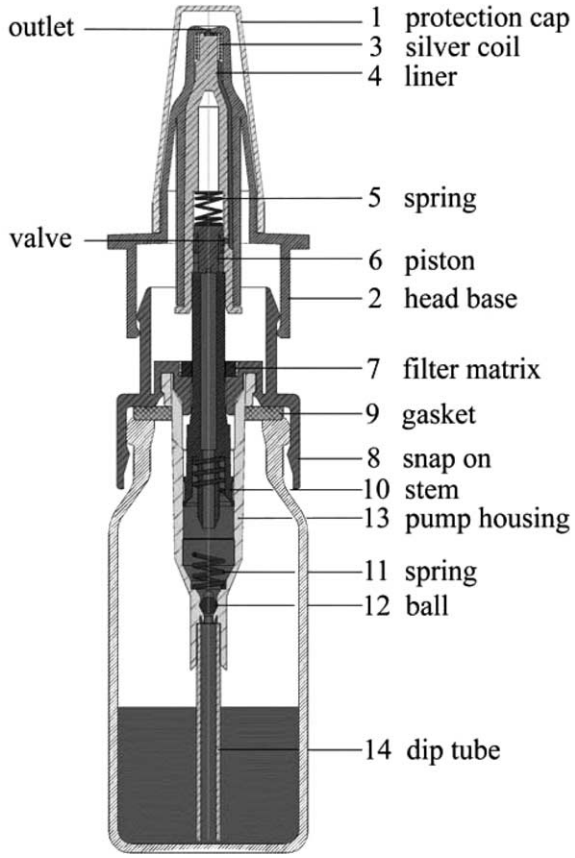

Fig. 1. Technical design of the 3K system.

(the space directly beneath the nozzle), the individual compartments of the system are physically separated from one another by means of valves, and the air which enters the system when the pump is used is channelled through a filter element (gasket, No. 9).

Preservative-free multi-dose containers are relatively new so that no methods for assessing the microbiological quality of these containers exist in the Ph. Eur. Additional literature was also missing. Consequently, we started to develop a number of novel test methods which provide relevant information in this respect [6]. Excellent microbiological quality has been shown for several preservative-free products in the 3K system over 6 weeks [7]. Therefore, it might be assumed that the 3K system allows even longer in-use stabilities for preservative-free products.

For an extension of the in-use stability of the open package, all microbiologically critical safety features of the 3K system must be tested in order to exclude instabilities and signs of wear. Possible risk factors could be the oligodynamic activity of the silver, the effectiveness of the valve and the quality of the filter element. In order to test the microbiological safety of an extension of the in-use stability up to several months, a new method, the in-use stability test that simulates patient use, has been established. The effectiveness of the combination of all relevant safety features should be examined carefully. If the containers still comply with the requirements of the Ph. Eur. this result might be an argument for an extension of the in-use stability from the microbiological point of view.

2. Materials and methods

2.1. Materials

Test organisms: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538P, *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404.

Media and buffer: NaCl–peptone (pH 7) (Ph. Eur.), Casein peptone soja peptone (caso) agar and sabouraud agar (Fluka Chemie GmbH, Switzerland). The nasal sprays tested were obtained from different manufacturers.

2.2. Methods

2.2.1. Incubation

Bacteria are incubated on caso agar for at least 18 h at 36 ± 1 °C. *C. albicans* and *A. niger* are incubated on sabouraud agar for at least 48 h at 20–25 °C.

2.2.2. Storing conditions

All samples are stored at room temperature

2.2.3. Preparation of the inoculum

The inoculum is prepared as described in the Ph. Eur., chapter 5.1.3. Colonies of the test organism are dispensed in NaCl–peptone buffer until the turbidity corresponds to the McFarland Standard 0.5 (about 1×10^8 CFU/ml).

2.2.4. Determination of viable counts (CFU/ml) within the first dose

After the last contamination and a 3-day storage period, one dose is discharged onto a caso agar plate and the viable count is determined after incubation. If growth occurs after 24 h of incubation, three more doses of the respective sample are tested in the same way.

2.2.5. Determination of viable counts (CFU/ml) within the contents

The body of the container is mechanically removed and the content is withdrawn under sterile conditions with the aid of a syringe. At least 2.5 ml of the contents are filtered, the filter is transferred to an agar plate and the viable count is determined following an appropriate period of incubation. If high cell counts occur, 1:10 dilutions of an aliquot of 50 μl of the remainder of the contents are prepared in NaCl–peptone buffer to allow counting. Fifty microliters of every dilution are transferred to caso agar plates and the viable count is determined in each case after an appropriate period of incubation.

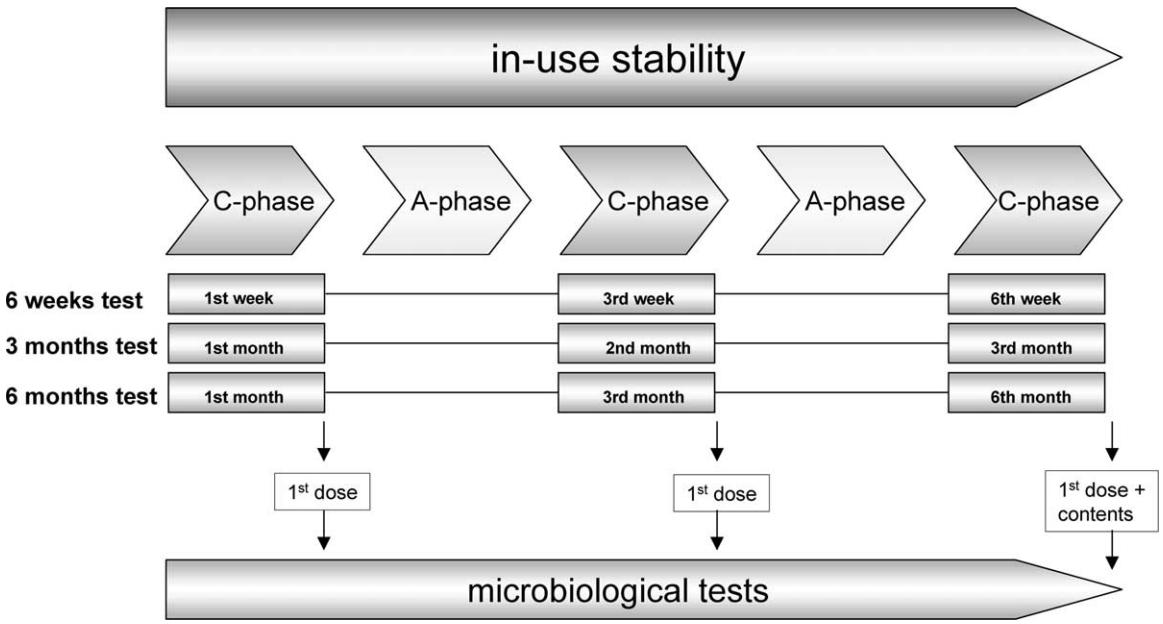

Fig. 2. Test schedule of an in-use stability test for 6 weeks, 3, and 6 months.

2.2.6. In-use stability

The aim of this test is to investigate, even under the critical conditions used, if (a) the system protects the contents from contamination, and (b) in all areas of the pump (especially those around the nozzle) microbial growth is prevented and the first dose delivered following contamination complies with the Ph. Eur. requirement of $\leq 10^2$ CFU/ml.

With respect to the planned in-use stability three contamination intervals (C-phase) are arranged regularly in a test schedule over the whole time period (Fig. 2). In addition to the C-phases, the contents of the containers are reduced via doses that are deposited into the air (A-phases) during the breaks between the C-phases. In order to simulate the lifecycle of a container the sum of all executed doses should represent at least 80% of the contents. The remaining content is necessary for microbiological testing.

The test methods were previously described in detail [6]: in the C-phase of the in-use stability test, potential contamination around the nozzle area is simulated by repeated dipping of the nozzle into a bacteria-containing suspension whilst discharging doses from the system. The inoculum of *P. aeruginosa* ATCC 9027 is diluted with NaCl–peptone buffer to obtain a suspension containing about 10^6 CFU/ml. The sample containers are used until a first dose is dispensed. All doses are discharged to waste. The containers are inverted and dipped into the bacterial suspension until the nozzle is submerged. They are then removed, whereupon one dose is discharged. The containers are then dipped into the suspension again and one dose is discharged into the suspension. The containers are removed once more and returned to the upright position, but the residual suspension adhering to the nozzle is not wiped off and the cap is not replaced. For a complete C-phase, this procedure is repeated twice daily for 4 days. After a C-phase the first dose is tested 3 days later. At the end of the testing period after the last C-phase the first dose as well as the contents are tested. The doses delivered in the A-phases are deposited in an upright position of the container into the air and collected in a receptacle.

2.2.7. Growth conditions in the drug and oligodynamic effect of silver

The oligodynamic effect describes the biocide effect of low concentrations of metals (mostly silver) against microorganisms The oligodynamic effect is dependant on the properties of the drug and the activity of the silver. In accordance with the test guidelines for the effect of antimicrobial preservatives (Ph. Eur. 5.1.3) the oligodynamic test monitors whether and to what extent the viable cell count is reduced and therefore microbiological growth is inhibited in the drug by the effect of silver within 28 days.

For each test organism and each point of time two samples are prepared: 10 μl of the inoculum are added to 990 μl of the preparation to be tested resulting in a final concentration of 10^5–10^6 CFU/ml. The samples are incubated with and without the silver coil at room temperature without light for 28 days. Following storage, the viable count is determined at defined $t = 0$, 6, 24 h, 7, 14, and 28 days by diluting 50 μl of each sample serially in NaCl–peptone buffer. The dilutions are transferred to agar plates and the viable count is determined in each case after appropriate periods of incubation.

Table 1
Microbiological quality of different nasalia in the 3K system following a 6-week in-use stability test

Product	*n*	CFU of 1st dose tested after						CFU/ml of contents after 3rd contamination in the 6th week	
		1st contamination in the 1st week		2nd contamination in the 3rd week		3rd contamination in the 6th week			
		<1	≥1	<1	≥1[a]	<1	≥1[a]	<0.4/ml	≥0.4/ml
A	20	20	0	18	2	19	1	20	0
B	20	20	0	19	1	18	2	20	0
C	20	20	0	20	0	20	0	20	0
Sum	60	60	0	57	3	57	3	60	0

n, Number of containers tested.
[a] In the first dose tested 1–3 CFU were found (one dose of products A, B and D contained 140 μl, one dose of product C contained 90 μl). Predominantly, airborne microorganisms were found, the test organism was found only in few cases. The next three doses tested the following day were free of microorganisms.

3. Results

3.1. In-use stability

Test schedules with C-phases interrupted by A-phases were worked out for four different products referring to the planned in-use stability. For each product the respective schedule had to include at least the longest in-use stability (product D) and additionally, might have included shorter time periods (products A–C). Therefore, three products (A–C) were tested with 20 containers each in a 6-week, a 3-month and a 6-month in-use stability test, product D was only tested in a 3-month test (Tables 1–3). The first dose delivered and the contents of all tested containers following the tests were microbiologically safe: the viable count was <1 CFU for the first dose and <0.4 CFU/ml for the contents. In a few cases the test organism and/or airborne microorganisms were detected within the first dose but this was comprehensible in view of the fact that the external orifice was exposed to the atmosphere throughout the entire test period and, deliberately, any wiping procedure was omitted. Therefore, the test organism as well as airborne microorganisms could have had adhered to the tip and were carried away by the next dose leading to the contamination. The next three doses tested the following day were found to be free from contamination in all cases supporting the thesis that any adhering microorganisms had been carried away by the doses dispensed before and that the contamination found did not derive from the inside of the container but only from the external orifice.

3.2. Growth conditions in the drug and oligodynamic effect of silver

The bactericidal and/or bacteriostatic properties of the pharmaceutical product, combined with the oligodynamic activity of silver was tested once for each product. The results for *P. aeruginosa* ATCC 9027 (as one example for the oligodynamic effect) are shown in Table 4 and Fig. 3a–d. This test simulated the conditions in the semi-exposed zone

Table 2
Microbiological quality of different nasalia in the 3K system following a 3-month in-use stability test

Product	*n*	CFU of 1st dose tested after						CFU/ml of contents after 3rd contamination in the 3rd month	
		1st contamination in the 1st month		2nd contamination in the 2nd month		3rd contamination in the 3rd month			
		<1	≥1	<1	≥1	<1	≥1	<0.4/ml	≥0.4/ml
A	20	20	0	20	0	20	0	20	0
B	20	20	0	20	0	20	0	20	0
C	20	20	0	20	0	20	0	20	0
D	20	20	0	20	0	20	0	20	0
Sum	80	80	0	80	0	80	0	80	0

Table 3
Microbiological quality of different nasalia in the 3K system following a 6-month in-use stability test

Product	*n*	CFU of 1st dose tested after						CFU/ml of contents after 3rd contamination in the 6th month	
		1st contamination in the 1st month		2nd contamination in the 3rd month		3rd contamination in the 6th month		<0.4/ml	≥0.4/ml
		<1	≥1[a]	<1	≥1	<1	≥1[a]		
A	20	19	1	20	0	19	1	20	0
B	20	20	0	20	0	19	1	20	0
C	20	20	0	20	0	20	0	20	0
Sum	60	59	1	60	0	58	2	60	0

[a] In the first dose tested 1–3 CFU were found (one dose of products A, B and D contained 140 μl, one dose of product C contained 90 μl). Predominantly, airborne microorganisms were found, the test organism was found only in few cases. The next three doses tested the following day were free of microorganisms.

below the orifice where the silver coil is located. The viable count of *P. aeruginosa* ATCC 9027 in the products A–C remained more or less constant over 28 days, whereas product D itself showed bactericidal properties. The presence of silver reduced the viable count effectively by at least 99.9% during the first 6 h in all four products. After 6 h (product D) and after 24 h (products A–C), the viable count decreased below the detection limit (<20 CFU/ml).

4. Discussion

The Ph. Eur. as well as the requirements for medical devices specify that nasalia should neither cause irritations nor have any adverse effects. Unfortunately, most common preservatives used in nasalia may impair the functioning of the nasal mucosa or the nasal cilia [2–4]. From the above mentioned requirements one can deduce that use of preservatives should be avoided whenever possible.

Ph. Eur. also specifies that the total viable aerobic count of nasal preparations should not exceed 10^2 CFU/ml. Preservative-free preparations dispensed using the 3K system must comply with this requirement in the same way as conventional preservative-containing preparations. Several tests assured microbiological safety over 6 weeks for different products using the 3K system [6,7]. The new in-use stability test should investigate the sustainable efficacy of all microbiological safety features of the 3K system over extended periods of simulated use. An extended time of use implies the possibility of signs of wear for each of the different safety mechanisms. The oligodynamic effect of silver might have been reduced over the time as a result of changing silver properties or changing milieu properties due to different evaporation characteristics of the product's ingredients. Even complete evaporation of the liquid in the semi-exposed zone of the tip could have resulted in a reduced silver activity caused by a salty coverage of the surface of the silver coil. Also sharp edges of grains of salt might have had an effect on the integrity of the valve. Furthermore, changing properties of the gasket itself or its precise incorporation into the system could have had an influence on its microbiological safety.

Following the in-use stability test, all microbiologically critical factors of safety withstood the extreme testing conditions. Neither the first dose nor the contents showed contaminations. Circumstances that might have influenced the safety features of the 3K system did not become evident

Table 4
CFU/ml in nasal preparations with and without silver during the oligodynamic effect over 28 days

CFU/ml in	Time (day)					
	0	0.25	1	7	14	28
Product A	5.0×10^6	2.3×10^6	1.6×10^6	2.0×10^6	4.1×10^6	6.6×10^6
Product A + silver	4.1×10^6	3.6×10^3	<20	<20	<20	<20
Product B	1.2×10^6	9.6×10^5	4.2×10^6	4.2×10^6	7.4×10^6	8.0×10^6
Product B + silver	1.0×10^6	2.0×10^2	<20	<20	<20	<20
Product C	1.3×10^6	7.4×10^5	7.2×10^5	2.4×10^5	8.6×10^5	2.2×10^5
Product C + silver	3.0×10^6	2.0×10^3	<20	<20	<20	<20
Product D	1.8×10^6	8.4×10^4	1.5×10^3	<20	<20	<20
Product D + silver	8.5×10^4	<20	<20	<20	<20	<20

Detection limit = 20 CFU/ml.

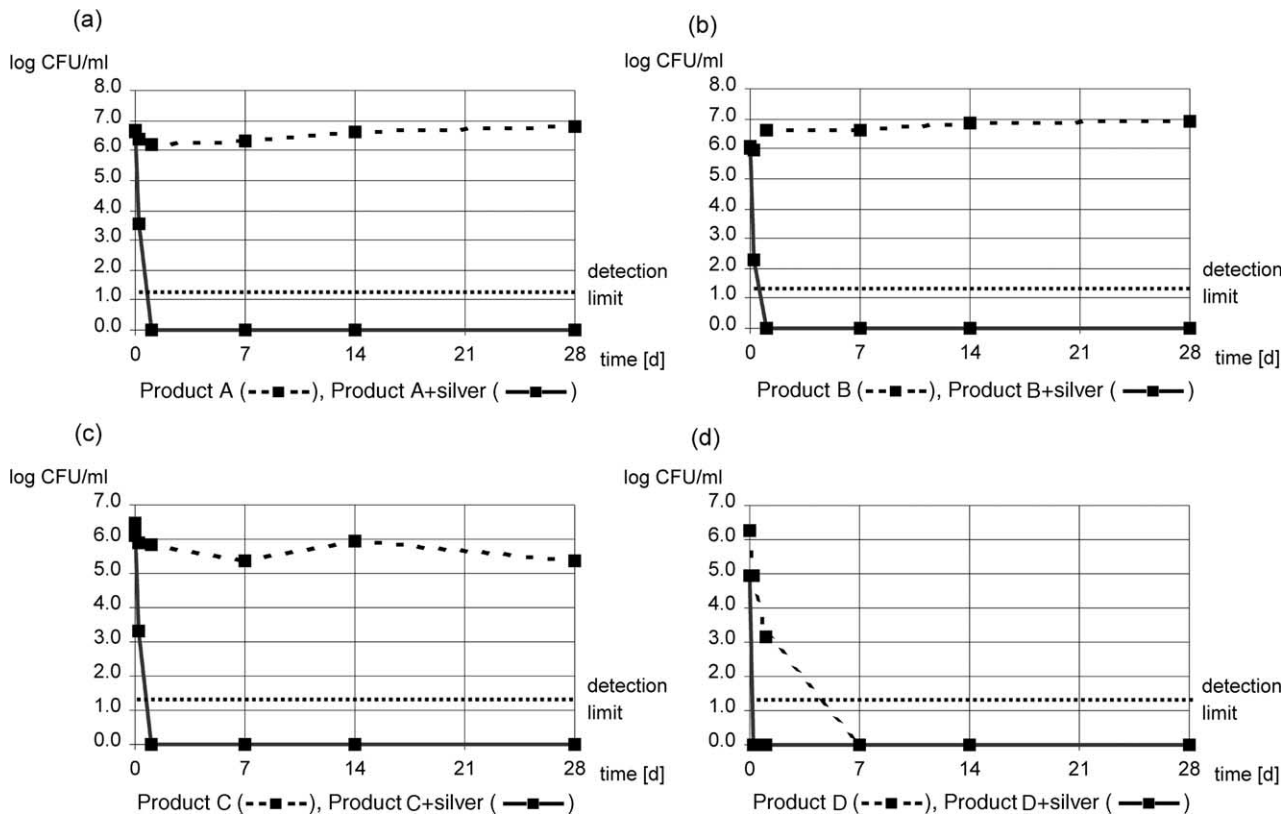

Fig. 3. Growth conditions and oligodynamic effect of silver in nasal preparation: (a) product A for *P. aeruginosa* ATCC 9027; (b) product B for *P. aeruginosa* ATCC 9027; (c) product C for *P. aeruginosa* ATCC 9027; and (d) product D for *P. aeruginosa* ATCC 9027.

with the described methods during the in-use stabilty test. All containers tested complied with the requirements of the Ph. Eur. and the first dose tested as well as the contents were microbiologically safe after 6 weeks as well as after 3 and 6 months.

These results demonstrate that, from a microbiological point of view, the 3K system meets all relevant criteria regarding the mechanical integrity of the system as a whole and the level of microbial contamination in the dead space. The technical design of the system, combined with the use of silver as an oligodynamic substance, ensures that the 3K system even over several months complies with the respective requirements for pharmaceutical drugs or medical devices, respectively.

In addition, these results verify certainty for the real use of many products that—even against the instruction leaflet—often extends 6 weeks and includes more than one patient.

Furthermore, it was demonstrated once again that compared to preserved products the use of a preservative-free system can be safe as well. The requirements of the Ph. Eur. can be met by preservative-free nasalia if the technical design comprises the respective constructional details. The possibility of preservative-free but microbiological by safe application of nasalia should be taken into account whenever innovations are developed. If nasal preparations are used long-term such as by patients with chronic diseases, allergies, or consumers in air-conditioned surroundings, the avoidance of preservatives seems to be a sustainable improvement of therapy [8]. Moreover, the application of systemic drugs via the nose in new indications (hormone therapy, analgesics, and motion sickness) might play a significant role in future developments.

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