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ABSTRACT

The development and validation of a product-specific test of filled glass ampoules for gas tightness by means of indicator reaction are described. Injectable medicinal products from the company Abnoba are produced under the exclusion of atmospheric oxygen, inert gassing by means of argon and with isotonic, isohydric ascorbate-phosphate buffer solution and filled into clear glass ampoules under inert gassing with argon gas. In case of damage, atmospheric oxygen penetrates the interior of the ampoule and leads to oxidation of the ascorbic acid and thus to a clearly visible, permanent discolouration of the ampoule contents to brownish, visible to the naked eye. In experiments with artificially damaged ampoules, it was shown that the gas exchange, i.e., the admission of atmospheric oxygen, which causes the brown discolouration of the ampoule contents, is temperature-dependent and lasted up to 12 days with cold storage (5 °C ± 3 °C) of the 1 ml and 10 ml ampoules, depending on the size of the crack. At room temperature, brown discolouration occurred more quickly, sometimes already the next day, mainly after 3–4 days. Based on these results, the waiting time, i.e., the minimum time required to test for discolouration of the ampoule contents, was set at 14 days with cold storage and subsequently introduced as a leak test for all ampoules containing ascorbate-phosphate buffer solution. No conclusions could be drawn about the size and depth of the cracks produced during these tests. Initial indications of a defect size of less than 10 µm were obtained based on microbiological tests of the contents of all brown-coloured defective ampoules found using this method. This is because the penetration of microorganisms is in principle only possible if the leakage is large enough for the liquid to enter. Gas penetration, therefore, does not necessarily lead to contamination of defective ampoules found using this method, as a review over a period of 12 years showed: that of 4 316 ampoules sorted out as defective during this period, 4 256 were evaluable. Only 4 times >100 colony-forming units (CFU) were found in the residual content, whereas 4 057 times 0 CFU were found, and in the remaining 195 ampoules between 99 and 1 CFU. The tested medicinal products have no antimicrobial properties.
To specify the defect size below 10 µm, tests with product-filled ampoules with built-in test leakage were carried out at Hilgenberg GmbH. These tests confirmed a defect size of <10 µm for this test method. However, problems with sealing the test leakage gas-tight in the ampoule meant that appropriately prepared product-filled glass capillaries had to be used for the further tests. In these tests, it could be shown that the defect size at which brown discolouration occurs is between 1 and 2 µm and thus in the same order of magnitude as the methods usually used for ampoules such as vacuum decay and high-voltage leak detection (HVLD). Furthermore, the waiting time of 14 days storage under refrigeration at 5 °C ± 3 °C determined in the initial process validation was confirmed.

KEY WORDS

- Leak Test
- Ampoules
- Gas Leak
- Indicator Reaction
- Oxidation
- Determination of Defect Size

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ZUSAMMENFASSUNG

Prüfung auf Gas-Dichtigkeit von gefüllten Glasampullen mittels Indikatormethode, Prüfung auf Oxidation des Inhalts

Beschrieben werden Entwicklung und Validierung einer produktspezifischen Prüfung von befüllten Glasampullen auf Gas-Dichtigkeit mittels Indikatorreaktion. Injektionsarzneimittel der Firma Abnoba werden unter Ausschluss von Luftsauerstoff, Inertbegasung mittels Argon und mit isotoner, isodohydrischer Ascorbat-Phosphatpuffer-Lösung hergestellt und unter Inertbegasung mit Argon in Klarglasampullen abgefüllt. Im Falle einer Beschädigung dringt Luftsauerstoff in den Ampulleninnenraum ein und führt zu Oxidation der Ascorbinsäure und damit einhergehend zu einer deutlich sichtbaren, mit bloßem Auge erkennbaren, dauerhaften

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Verfärbung des Ampulleninhalts ins Bräunliche. In Versuchen mit künstlich beschädigten Ampullen wurde gezeigt, dass der Gasaustausch, also der Zutritt von Luftsauerstoff, der die Braunverfärbung des Ampulleninhalts bewirkt, temperaturabhängig ist und bei Kühllagerung (5 °C ± 3 °C) der 1-ml- und 10-ml-Ampullen je nach Größe des Risses bis zu 12 Tagen dauerte. Bei Raumtemperatur trat Braunverfärbung schneller ein, z. T. bereits am nächsten Tag, zumeist nach 3–4 Tagen. Auf Grund dieser Ergebnisse wurde die Wartezeit, also die Mindestdauer bis zur Prüfung auf Verfärbung des Ampulleninhalts auf 14 Tage bei Kühllagerung festgelegt und in der Folge als Dichtigkeitsprüfung aller Ascorbat-Phosphat-Pufferhaltigen Ampullen eingeführt. Aussagen über Größe und Tiefe der bei diesen Untersuchungen erzeugten Risse konnten nicht gemacht werden. Erste Hinweise auf eine Defektgröße von unter 10 µm ergaben sich auf Grundlage mikrobiologischer Prüfungen des Inhalts aller mit diesem Verfahren gefundenen braunverfärbten defekten Ampullen. Denn das Eindringen von Mikroorganismen ist prinzipiell nur dann möglich, wenn die Leckage so groß ist, dass Flüssigkeit eindringen kann. Gasdurchtritt führt somit nicht zwangsläufig zu einer Verkeimung von mit diesem Verfahren gefundenen Defektampullen, wie ein Review über einen Zeitraum von 12 Jahren zeigte: Von 4 316, in diesem Zeitraum als defekt aussortierten Ampullen waren 4 256 Ampullen auswertbar. Lediglich 4-mal wurden im Restinhalt >100 Kolonie bildende Einheiten (KBE), dagegen 4 057mal 0 KBE, in den restlichen 195 Ampullen zwischen 99 und 1 KBE gefunden. Die geprüften Arzneimittel haben keine antimikrobiellen Eigenschaften.
Zur Präzisierung der unter 10 µm liegenden Defektgröße wurden Versuche bei der Firma Hilgenberg GmbH mit produktbefüllten Ampullen mit eingebauter Testleckage durchgeführt. Diese Versuche bestätigten eine <10 µm liegende Defektgröße dieses Prüfverfahrens. Jedoch führten Probleme, die Testleckage gasdicht in der Ampulle zu verschließen, dazu, dass für die weiteren Versuche entsprechend präparierte produktbefüllte Glaskapillaren verwendet werden mussten. In diesen Versuchen konnte gezeigt werden, dass die Defektgröße, bei der Braunverfärbung eintritt, zwischen 1–2 µm und damit in der gleichen Größenordnung wie die üblicherweise für Ampullen gebräuchlichen Verfahren wie Unterdruckabfall- (Vacuum Decay) und Hochspannungs-Lecktest (High-voltage leak detection, HVLD) liegt. Ferner wurde die im Rahmen der anfänglichen Verfahrensvalidierung ermittelte Wartezeit von 14 Tagen Lagerung unter Kühlung bei 5 °C ± 3 °C bestätigt.

1. Introduction

The integrity of tightly closed, filled ampoules is a basic requirement for maintaining product quality.

There are numerous causes of leaks. These include, for example, quality defects of the empty ampoules used, thermally induced cracking, e.g., when melting or melting the ampoule skewer after filling the ampoule, as well as transport-related damage. The latter can happen during the separation of the ampoules in filling, visual inspection, and labelling machines, due to dynamic pressure in buffer zones in these machines, in any transport of the ampoules, including the finished products to the patient.

Holes and cracks created in this way, if not detected, allow subsequent leakage of the contents as well as the penetration of germs and atmospheric oxygen. This leads to spoilage of the products and endangers the patients.

The testing of ampoules for leak tightness or integrity is therefore also a regulatory requirement. Tightness must be ensured over the entire life cycle of the medicinal product. The European Pharmacopoeia [1] only requires that the tightness of the containers be ensured appropriately. Annex 1 of the EC GMP Guideline [2], which has long stipulated a corresponding 100 % test for ampoules, is more precise. The new version of Annex 1, which has not yet entered into force, additionally states that a visual inspection, such as is carried out when testing for visible particles, is not to be considered an acceptable integrity test method on its own [3]. Worldwide – from the Parenteral Drug Association (PDA) Technical Report No. 27 from 1998 [4] to the current USP (The United States pharmacopoeia) with its Chapter 1207 "Container Closure Integrity (CCI) Testing" [5] – there is no set of regulations that prescribes a specific testing method of ampoules. The responsibility for choosing a suitable test method lies with the pharmaceutical manufacturer. The selection is based on the properties of the primary packaging material and the filled product, as well as the resulting specifications. The main risk in the event of an ampoule defect is certainly microbial contamination of the sterilised contents.

1.1 Methods for Integrity Testing of Ampoules

Known methods for testing for water and bacterial tightness are blue bath testing and microbiological immersion testing. Both methods have low sensitivity. In addition, blue bath testing involves the risk of undetected defective, slightly discoloured ampoules, especially if the ampoule contents themselves are coloured. Therefore, these procedures only serve as a reference method in the validation of more sensitive integrity tests. [6,7,8]. The precision of blue bath testing is at hole diameters of 15–30 µm [9]. Knebel and List [6] described in their experiments that hole lumens of <10 µm are outside the detection limit of the test in blue bath testing.

Much more sensitive are methods that detect the passage of gas and thus find defective ampoules. Methods frequently used for ampoules, which are reliable, non-destructive and produce a reproducible result in a reasonable test time in routine testing, are vacuum decay and High-voltage leak detection (HVLD). These integrity tests are mainly performed in the range of 2–20 µm defect size. This also means that the bad ampoules found in this way do not necessarily have to be contaminated. This is because the penetration of microorganisms is in principle only possible if the leakage is so large that liquid can enter. For example, Krebsbach et al. [7] deter-

Table 1

Leaking (brown discoloured) ampoules (without exception abnobaVISCUM) detected in microbiological tests from May 2005 to Jan 2022.

Number of leaking Ampoules	Result CFU/Ampoule Contents
60	Dried up contents
3	Overgrown culture medium
1	101
17	99–10
56	9–2
122	1
4 057	0
4 316	Total Number

mined a detection limit of 10 µm diameter in microbiological immersion tests for glass vials with different test leakages.

1.2 Development of a product-specific Process and Initial Validation

abnobaVISCUM® and Betula Folium ABNOBA, injectables filled in clear glass ampoules, are prepared to the exclusion of atmospheric oxygen, inert gassing by argon gas and the addition of ascorbic acid, an antioxidant as a component of the aqueous extraction and dilution medium [10]. Oxidation protect, as well as the maintenance of sterility are among the essential tasks of the primary packaging of these drugs.

Ampoules of abnobaVISCUM of mg strengths and Betula Folium D3 ABNOBA contain isotonic and isohydric ascorbate-phosphate buffer solution [11]. Without inert gassing, such drug solutions turn brown in the presence of atmospheric oxygen. This property is the basis for the development of a product-specific leak test. In the event of damage, a crack or hole, atmospheric oxygen penetrates the interior of the ampoule and leads to oxidation of the ascorbic acid and thus to a clearly visible, permanent discolouration of the ampoule contents to brown, visible to the naked eye.

Within the framework of an initial validation, it was to be determined how long it takes until such a brown discolouration is visually recognisable. For this purpose, the glass of filled ampoules was selectively heated with a Bunsen burner flame and then the formation of cracks was produced by dripping cold water on them. The ampoules artificially damaged in this way were stored at different temperatures and the length of time until the first brown discolouration was determined. The gas exchange, i.e., the influx of atmospheric oxygen, which causes the brown discolouration of the ampoule contents, is temperature-dependent and lasted up to 12 days with cold storage (5 °C ± 3 °C) of the 1 ml and 10 ml ampoules, depending on the size of the crack. At room temperature, brown discolouration occurs more quickly, sometimes the very next day, mainly after 3–4 days. Based on these results, the waiting time, i.e., the minimum duration until testing for discolouration of the ampoule contents, was set at 14 days with cold storage (5 °C ± 3 °C) and subsequently introduced as a leak test for all ampoules containing the ascorbate-phosphate buffer solution.

This test for brown discolouration due to air ingress is a non-destructive 100 % test for gas tightness. For this purpose, each bulk goods box containing approx. 500 units of 1 ml and approx. 190 units of 10 ml ampoules are opened and viewed from different sides and angles under suitable lighting conditions. In this way, even the slightest differences in colour can be detected well and quickly and defective ampoules can be found and sorted out.

However, it was not possible to make any statement about the size and depth of the cracks produced during these examinations or whether the cracks were continuous at all. In individual defective ampoules produced in this way, cracks were visually recognisable, but brown discolouration did not occur. The cracks were not complete and there was no gas exchange. These ampoules remained leak-proof despite visible cracks, and their contents did not discolour. In daily operation, however, such ampoules will stand out in another 100 % test, namely the test for visible particles [12], in which not only ampoules containing particles but also those with glass defects of any kind are sorted out.

1.3 Accompanying Microbiological Tests

In the period from May 2005 to Jan 2022, 4 316 defective ampoules were detected by means of this product-specific test for brown discolouration due to air ingress (table 1). The rate of leaking ampoules was well below 0.02 %, which is within the expected range for a leak test. All ampoules sorted out as leaking in this way during this period were microbiologically tested for the presence of aerobically growing microorganisms according to 2.6.12 Ph. Eur. [13]. For the most part, – 4 057 times – no germs were found in the residual filling of the ampoules, only 4 times germ counts above 100 CFU (colony-forming units)/ampoule content and 17 times germ counts above 10 CFU/ampoule content. With these low bacterial counts, one cannot speak of a high risk of contamination of defective ampoules. The tested medicinal products have no antimicrobial properties. This shows that the contents of ampoules only become unsterile extraordinarily rarely (<0.1 %) due to leakages that allowed atmospheric oxygen to penetrate (and were

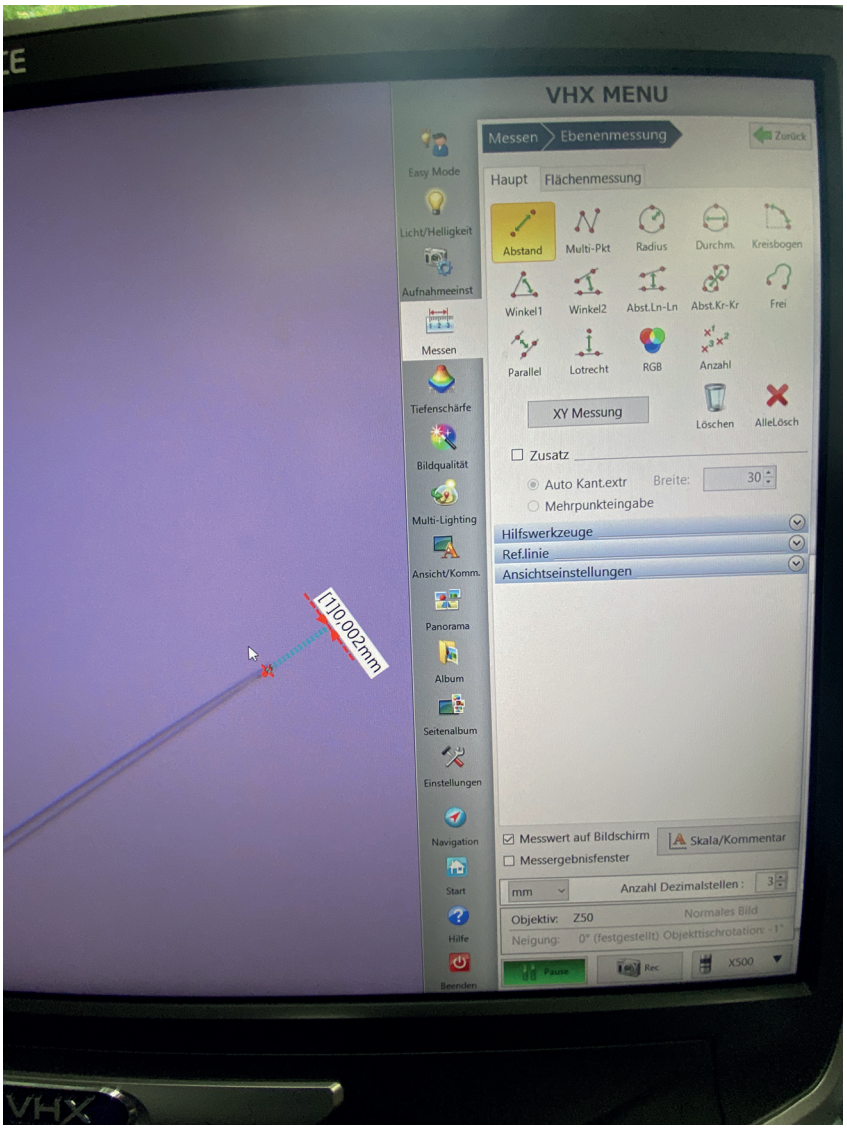

Figure 1: Measurement image from the VHX6000 digital microscope, Keyence, under which the tips of the drawn capillaries were measured (source of all figures: the authors).

thus detected). This unsterility therefore only occurs in all probability if the defects also have a corresponding hole size of >10 µm (see chapter *Methods for integrity testing of ampoules*). This gives the first indication that the product-specific test for air leakage we performed has a defect size below 10 µm. In the following, we tried to determine this defect size more precisely and to check the specified waiting time of 14 days with cold storage (5 °C ± 3 °C).

2. Material and Methods

For the determination of defect size and waiting time, product-containing glass containers (ampoules or glass capillaries) with defined holes were prepared under exclusion of oxygen and argon protection and stored cool (5 °C ± 3 °C) and at room temperature (25 °C ± 2 °C) until the brown discolouration of the contents and beyond. Temperature and humidity during storage were monitored.

Part 1: Experiments with Ampoules

For this purpose, a method developed by Hilgenberg GmbH was available [14].

Perforated discs with known hole diameters (e.g., 40 µm, 20 µm, 10 µm, 5 µm and smaller) were produced from glass capillaries in advance. Holes large enough to allow filling with the product under oxidation protection (evacuation and inert gassing with argon gas) were created in the bottom of empty 1 ml burn-on ampoules by means of a laser and subsequent relaxation of the glass. The ampoules were filled with abnobaVISCUM Fraxini 20 mg, a green-yellow coloured mistletoe extract. After filling, the ampoules were sealed with the prepared perforated discs described above or glass discs of the same size but without a hole (to control filling and resealing under oxidation protection). This was done using an adhesive that was further hardened by means of UV light. The test vials prepared in this way were stored at room temperature and in the refrigerator and the time when brown discolouration occurred was determined visually. Testing for brown discolouration of the ampoule contents is carried out visually by trained personnel. The ampoules should discolour within 14 days, depending on the size of the hole and the storage temperature, and the ampoules without a hole should not discolour. Duration of storage: fixed upside down, up to 4 weeks.

Mistletoe extract: abnobaVISCUM 900 (Fraxini 20 mg) of batches 509A03 and 901A20, filled in 10 ml glass ampoules.

Glass ampoules: Clear neutral glass of hydrolytic class I [15], tension-free closed 1 ml OPC burn-on ampoules (form D) with red colour marking. The ampoules comply with the specifications of DIN EN ISO 9187-1 and DIN EN ISO 9187-2 (breaking strength),

Adhesives: Vitralit 1605, 1702 and 4050 (different viscosity): UV-curing adhesives from Panacol-Elosol GmbH.

The ampoules were filled and sealed in a glove box (Captair® Pyramid with 2 glove ports, VWR No. 135-1734), the gas chamber of which was permanently flushed with argon gas 4.6. After checking the gas chamber for the absence of air, the ampoules were individually pre-purged with argon, filled with product and post-gassed. Around the prepared perforated discs, the gel-like adhesive was applied evenly and, in several layers, fixed and hardened so that the filling opening was sealed. Outside the glove box, a 15-minute post-curing was carried out in a UV chamber.

Part 2: Experiments with Glass Capillaries

These experiments were also carried out at Hilgenberg. Instead of subsequently providing ampoules with holes of a defined size, prepared glass capillaries were used for this purpose. These glass capillaries were drawn in such a way that a tip with the desired inner diameter or completely closed was created. When filling them with ascorbic acid-containing mistletoe extract under oxidation protection (see above), care was taken that the extract did not touch the prefabricated hole

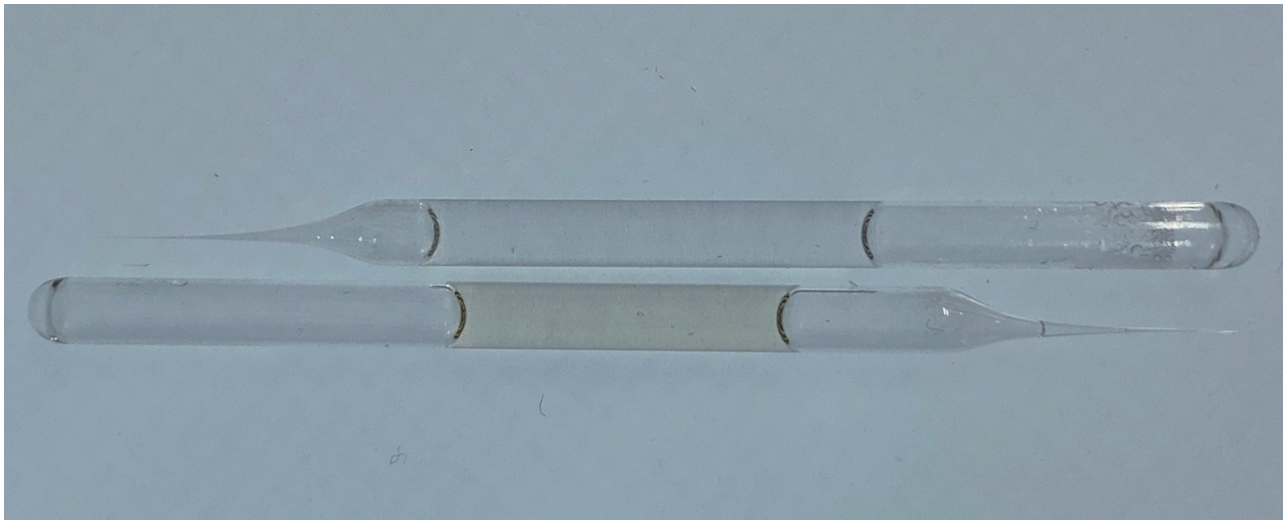

Figure 2: Capillary with brown discoloured content due to air ingress (bottom) and an undamaged completely sealed capillary with non-discoloured content (top).

due to its surface tension. At a sufficient distance from the product filling, the capillary was melted on the opposite side and thus permanently sealed without glue.

Container material: Glass capillaries made of borosilicate glass 3.3, [16] ends cut.
L = 100 ± 0.5 mm
Outer = 2.5 ± 0.1 mm
Inner = 2.0 ± 0.1 mm
Wall thickness = 0.25 ± 0.05 mm

Extending the capillaries to a defined diameter of the opening:

Pick-up of the glass capillaries on an automated lathe in 2 collets, central heating until the beginning of softening of the glass, pulling apart with a fast pull, central scratching with a diamond wire and separation. Capillaries with a completely closed tip were also produced. The newly formed tips were then measured under a digital microscope (VHX6000, Keyence; fig. 1) to see whether the inner diameters had the target size or whether the tip was completely closed.

Filling and closing of the capillaries are prepared in this way:

Work in the previously described glove box under continuous argon gas supply:

- Approximately 30 sec Pre-purge the capillaries with argon gas,
- Filling of the capillaries, lying down, without wetting the capillary ends,
- Close the filling side of the capillaries with an EVA (ethylene vinyl acetate) stopper.
- Removing the capillaries from the glove box. Reason: The glove box and the sealing machine are located in different production areas.
- The capillaries are picked up again in the collet chuck of the sealing machine and the rear end is melted through.

Storage of the capillaries and control for brown discolouration: as previously described in part 1 but lying down. In addition, storage was in the dark at both temperatures. Duration: 4 weeks starting on the day of filling. Early termination was possible in case of clear test results and no expected changes. It was also possible to extend the test if necessary.

The test for brown discolouration of the capillary content (fig. 2) was carried out visually every working day by trained personnel.

3. Results

3.1 Part 1: Experiments with Ampoules

Table 2 shows an example of the result of an experiment in which 1 ml OPC burn-on ampoules – as described under *Material and methods* – were subsequently filled with abnobaVISCUM Fraxini 20 mg and closed by means of glass discs with different hole diameters and sealed gas-tight with adhesive. The storage was carried out over 26 days cool and at room temperature.

Through this experiment, the following could be shown:

- Oxidation of the ascorbic acid also occurs with hole diameters below 10 µm,
- defective ampoules are visually recognisable after less than 14 days of cool storage.
- the speed of brown discolouration is independent of the hole diameter and occurs much more slowly with cool storage than at room temperature.

However, there were 6 of a total of 30 ampoules that did not discolour, although this would actually have been expected. In these ampoules, the adhesive had not only sealed the filling opening but, before it could dry, had flowed to the underside of the perforated disc, and subsequently sealed the hole there with a defined diameter. This prevented air from entering and leading to oxidation of the ascorbic acid, and thus to brown discolouration of the ampoule contents.

Such outliers occurred in all test series, unpredictably and to varying degrees. It also happened that the amount of adhesive was insufficient or shrunk so that there were other holes in addition to the hole in the disc. Thus, ampoules that were not supposed to discolour discoloured unpredictably.

■ **Table 2**

1 ml empty ampoules (OPC burn-on ampoules) subsequent filled with abnobaVISCUM Fraxini 20 mg and sealed by means of glass discs with different hole diameters and sealed with adhesive. Storage for 26 days in a cool place at room temperature.

Hole diameter [µm]	Cool storage	Storage at room temperature
	Time (days)* until the visible brown discolouration	
0	No discolouration	No discolouration
0	No discolouration	No result
5	9–11	1–4
5	9–11	12
5	9–11	No discolouration
10	9–11	1–4
10	9–11	1–4
10	9–11	No discolouration
10	9–11	No discolouration
20	No discolouration	1–4
20	No discolouration	1–4
20	9–11	No discolouration
20	9–11	1–4
40	9–11	1–4
40	9–11	1–4

*All readings were made on weekdays, not weekends, holidays, and bridging days. Therefore, the brown discolouration could also have occurred a few days earlier, and in such cases, a corresponding period is indicated in the table.

To solve these problems, the following variants were tested, among others:

- Longer capillaries with a defined diameter were used instead of perforated discs.
- Adhesives of different viscosities.

Unfortunately, it was not possible to solve the problems in such a way that the results of such a series of experiments could be confirmed in a repeat test.

Conclusion: Closing the filling hole of filled empty ampoules gas-tight by glueing on a prefabricated glass pane is currently no suitable process or adhesive available. Therefore, the experiments with ampoules were terminated and further tests were carried out exclusively with glass capillaries.

3.2 Part 2: Experiments with Glass Capillaries

The glass capillaries could be provided with defined holes, filled, and sealed gas-tight much better than was possible with ampoules. Nevertheless, there were 2 outliers in the test series from June 2021. The long time until brown discolouration in capillaries with 10 µm holes and cool storage cannot be explained, especially since all other capillaries with pre-prepared holes discoloured in a significantly shorter time.

Apart from that, the results of both test series summarised in table 3 not only confirm that defective containers are visually recognisable after less than 14 days in cold storage, but in addition, the defect size below 10 µm could be specified. A defect size between 1–2 µm was found.

4. Discussion and Conclusion

Due to the absence of massive microbial growth in the leaking ampoules discarded over 16 years, a defect size of less than 10 µm hole diameter can be assumed for the product-specific test for gas tightness by means of brown discolouration described here.

Unfortunately, the defect size could not be determined precisely enough with product-containing ampoules. This is because it was not possible to reproducibly seal the filling hole of empty ampoules gas-tight after they had been filled with the product by glueing on prefabricated glass discs with holes of known size.

However, this was possible with appropriately prefabricated glass capillaries.

In these experiments, it could be shown that the defect size at which brown discolouration occurs is between 1 and 2 µm and thus in the same order of magnitude as the methods usually used for ampoules, such as vacuum decay and HVLD. Furthermore, the waiting time

Table 3

Glass capillaries prepared with defined holes subsequent filled with abnobaVISCUM Fraxini 20 mg and sealed gas-tight. Storage cool and at room temperature. Storage time test run 1: 23 days; test run 2: 18 days.

Hole diameter [µm]	Capillary-no.	Time (days)* until the visible brown discolouration			
		Cool storage		Storage at room temperature	
		Test run 1	Test run 2	Test run 1	Test run 2
0	1	No discolouration of contents within the test period			
	2				
	3				
	4				
	5				
1	1	–	No discolouration of contents within the test period	–	6–8
	2	–		–	6–8
	3	–		–	6–8
	4	–		–	6–8
	5	–		–	6–8
2	1	7–11	–	2–4	–
	2	7–11	–	7–11	–
	3	7–11	–	2–4	–
	4	7–11	–	2–4	–
	5	7–11	–	7–11	–
5	1	7–11	12	2–4	6–8
	2	7–11	12	2–4	6–8
	3	2–4	12	2–4	6–8
	4	2–4	12	12	6–8
	5	2–4	12	12	6–8
10	1	16–19	12	2–4	6–8
	2	16–19	12	2–4	6–8
	3	–	12	–	6–8
	4	–	12	–	6–8
	5	–	12	–	6–8
completly open capillaries without tip	1	–	6–8	–	1
	2	–	6–8	–	1
	3	–	6–8	–	1
	4	–	6–8	–	1
	5	–	6–8	–	1

*All readings were made on weekdays, not weekends, holidays, and bridging days. Therefore, the brown discolouration could also have occurred a few days earlier, and in such a case, a corresponding period is indicated in the table.
– = not tested.

of 14 days storage under refrigeration at 5 °C ± 3 °C determined during the initial procedure validation was confirmed.

Compared to all other leak testing methods, the method described and tested here has the advantage that even patients can detect defective ampoules shortly before use due to the visually recognisable brown discolouration. This means that even defects that can sometimes still occur during transport become visible. Furthermore, no complex testing machine is required. The method works with any indicator contained in the drug solution that makes the presence of oxygen visible, not

only, as in our case, with isotonic, isohydric ascorbate-phosphate buffer solution. This could already be considered in the galenic development of an injection remedy and, if the production is carried out under oxygen exclusion, one gets a safety and easy-to-use leak test method that does not require much equipment.

This provides evidence of the leak tightness of ampoules containing ascorbate-phosphate buffer solution based on their product properties over the entire life cycle of the medicinal product – as required by regulation. This is because the gas-tightness test used for this purpose can detect leaks from the time the ampoules are sealed until they are used on the patient with a defect size of 1–2 µm.

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