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Longterm physicochemical stability of ready-to-administer human insulin injection solutions 1 I.U./mL in 50 mL plastic syringes

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Abstract

Objectives: The objective of this study was to investigate the physicochemical stability of ready-to-adminster human insulin (HI) 1 I.U./mL injection solutions of two different brand products diluted with 0.9% NaCl solution under different storage conditions either in 50 mL disposable plastic syringes or as bulk solution in infusion bags.
Methods: HI test solutions 1 I.U./mL were prepared with Huminsulin® Normal 100 and Actrapid® Penfill®, diluted with 0.9% sodium chloride infusion solution, and filled in Original-Perfusor® syringes or BD® Perfusion syringes. Test solutions were stored for 90 days at 2–8 °C/dark or at 20–25 °C/diffuse room light. Bulk solutions 1 I.U./mL prepared with Huminsulin® Normal 100 were stored in two different 3 L infusion bags (Ecobag® click and ExactaMix® EVA bag) at 2–8 °C/dark for five days. HI concentrations were determined by reversed-phase high-performance liquid chromatography at predefined time points. Beside regular visual inspection, subvisible particles and pH values were measured.
Results: Ready-to-adminster Huminsulin® Normal and Actrapid® Penfill® injection solutions 1 I.U./mL prepared in 50 mL Original-Perfusor® syringes or BD® Perfusion syringes remained physicochemically 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. Prefilled 3 L polyolefin infusion bags (Ecobag® click) are suitable for the preparation of Huminsulin® 1 I.U./mL bulk solutions. In ethylene vinyl acetate (EVA) bags, HI concentrations decreased rapidly.
Conclusions: Ready-to-administer Huminsulin® Normal and Actrapid® Penfill® injection solutions 1 I.U./mL can be prepared in advance by dilution with 0.9% sodium chloride infusion solution and filled into 50 mL Original-Perfusor® syringes or BD® Perfusion syringes; subsequent storage at 2–8 °C/dark is possible for up to 90 days. For preparation of bulk solutions, the prefilled polyolefin infusion bag is appropriate.

Keywords: continuous intravenous injection; EVA infusion bag; human insulin 1 I.U./mL; physicochemical stability; plastic syringe; polyolefin.

Introduction

Diluted human insulin (HI) solutions 1 I.U./mL are administered to intensive care patients by continuous injection to treat hyperglycemic status. The ready-to-administer (RTA) HI injection solutions are either reconstituted on the ward or prepared by pharmacy-based central intravenous additives services using multidose vials of HI as starting material and 50 mL plastic syringes as primary containers [1, 2]. There are three licensed medicinal products marketed in Germany which can be used as starting material: Huminsulin® Normal 100 (Lilly Deutschland GmbH) [3], Actrapid® Penfill® (Novo Nordisk A/S) [4], and Insuman® Rapid (Sanofi-Aventis Deutschland GmbH) [5]. Each solution for injection contains 100 I.U./mL HI in a multidose vial or cartridge. Table 1 shows the product characteristics of Huminsulin® Normal and Actrapid®, both described here. Details regarding the product characteristics of Insuman® Rapid and the stability of the 1 I.U./mL RTA injection solution have already been published in this journal [6]. Apart from that, only the stability of RTA Actrapid® 1 I.U./mL in 60 mL BD PlastiPak™ syringes under various temperature conditions over a maximum period of seven months is reported in the literature [7].

It is wellknown that chemical degradation of the peptidic HI is characterized by deamidation and the formation of covalent dimers and higher order polymers [8, 9]. Storage temperature, pH and the excipients used in the formulation mainly influence the chemical degradation. To

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Table 1: Product characteristics of EMA approved human insulin injection solutions 100 I.U./mL.

	Huminsulin® Normal 100 [3]	Actrapid® Penfill® [4]
	Lilly Deutschland GmbH	Novo Nordisk A/S
Active substance	Human insulin produced by recombinant DNA technology in *E. coli*	Human insulin produced by recombinant DNA technology in *Saccharomyces cerevisiae*
Excipients	Metacresol (Ph. Eur.) Glycerol Water for injection	Zinc chloride Glycerol Metacresol (Ph. Eur.) Sodium hydroxide (for pH adjustment) Hydrochloric acid (for pH adjustment) Water for injection
pH	7.0–7.8 [17]	7.0–7.8 [17]

stabilize discrete insulin hexamers and to prevent aggregation, insulin containing medicinal products are formulated with m-cresol and zinc ions [8–10]. Changes in pH, exposure to elevated temperatures, agitation, and/or contact with hydrophobic surfaces induce conformational changes that promote aggregation, formation of insulin fibrils and precipitates [8–10]. In addition, HI is associated with adsorption phenomena to various materials, including primary containers, tubings and filters of administration systems made of glass, polyvinyl chloride (PVC), ethylene vinyl acetate (EVA), polyethylene (PE), and other polyolefin plastics [7, 11–16]. HI adsorption is influenced by surface area, temperature and the presence of proteins (e.g. human albumin), amino acids, glucose, and electrolytes [11].

The objective of this study was to determine the physicochemical stability of 1 I.U./mL HI injection solutions of two different brand products (Huminsulin® Normal 100, Actrapid® Penfill®) diluted with 0.9% sodium chloride solution in two types of 50 mL disposable syringes (Original-Perfusor® syringe B.Braun, BD® Perfusion syringe) as primary packing material and stored at different conditions for up to 90 days. Simulating the pharmacy-based, aseptic preparation of such syringes in batches, compatibility of Huminsulin® Normal 1 I.U./mL bulk solution with ethylene-vinyl acetate (EVA) or polyolefin (PO) infusion bags was investigated.

Materials and methods

Chemicals and reagents

Mobile phase: Water HPLC grade (Applichem GmbH Darmstadt, Germany; LOT: 0X011438), phosphoric acid 85% HPLC grade (Sigma-Aldrich Chemie GmbH, Steinheim, Germany; LOT: BCCC4809), Disodiumphosphate-monohydrate HPLC-Grade (Fisher Scientific, Loughborogh, UK; LOT: 1923632), acetonitrile for HPLC (Honeywell International Inc., Seelze, Germany; LOT: K0840); Insulin injection solutions: Huminsulin® Normal 100, 100 I.U./mL solution for injection in a vial, 10 mL (Lilly Deutschland GmbH, Bad Homburg, Germany; LOT: D143929, expiry date: 02/2022), Actrapid® Penfill® 100 I.U./mL, solution for injection in cartridge, 3 mL (Novo Nordisk A/S, Bagsvaerd, Denmark; LOT: JR71R57, expiry date: 12/2021); Vehicle solutions: Pre-filled 0.9% sodium chloride 250 mL freeflex® infusion bags (Fresenius Kabi; LOT: 13PCF121, expiry date: 02/2023), pre-filled 0.9% sodium chloride B. Braun Ecobag® click, 3,000 mL (B.Braun Melsungen AG, Melsungen, Germany; LOT: 203527671, expiry date: 07/2022).

Primary packaging material and devices

Original-Perfusor® syringe 50 mL (B. Braun Melsungen AG, Melsungen, Germany; LOT: 20D2282001),
50 mL BD® Perfusion syringe (BD, Becton, Dickinson and Company limited, Louth, Ireland; LOT: 1710270),
Combi-Stopper (B.Braun Melsungen AG, Melsungen, Germany; LOT: 20DO5A8717);
ExactaMix® EVA bag – parenteral 3,000 mL (Baxter Healthcare SA, Zurich, Switzerland; LOT: L061CF),
B. Braun Ecobag® click, 3,000 mL (B.Braun Melsungen AG, Melsungen, Germany; LOT: 203527671, expiry date: 07/2022),
Injekt®-F 1 mL Luer Solo syringe (B.Braun Melsungen AG, Melsungen, Germany; LOT: 20E04C8),
Microlance™ 3, 18G × 1½″ (BD, Becton, Dickinson and Company Limited, Louth, Ireland; LOT: 200410),
Screw top vials 1.5 mL, 32 × 11.6 mm (MZ-Analysentechnik GmbH, Mainz, Germany).

Preparation of HI test solutions in plastic syringes

Diluted Huminsulin® Normal test solutions were prepared in Original-Perfusor® syringes 50 mL or 50 mL BD® Perfusion syringes as primary containers by adding 0.5 mL HI 100 I.U./mL concentrate to 49.5 mL 0.9% NaCl solution withdrawn from 250 mL freeflex® infusion bags. Thereby a nominal HI concentration of 1 I.U./mL was achieved. Barrel and plunger of both syringe types are composed of polypropylene (PVC- and latex-free), the plunger stopper of synthetic rubber; silicone oil is used as lubricant. Diluted Actrapid® Penfill® test solutions were prepared likewise in Original-Perfusor® syringes 50 mL as primary containers.

Preparation of HI bulk solutions

HI 1 I.U./mL bulk solutions were prepared by adding 30 mL Huminsulin Normal 100 I.U./mL (withdrawn from three vials by a 50 mL syringe) either to a pre-filled 0.9% NaCl 3 L B. Braun Ecobag® click polyolefin infusion bag or to an ExactaMix® 3 L EVA (ethylene vinyl acetate) infusion bag. Prior to this, the overfill plus an additional volume of 30 mL 0.9% NaCl solution were withdrawn from the pre-filled polyolefin infusion bag. The empty EVA-bag was filled with 2,970 mL 0.9% NaCl infusion solution, using the ExactaMix™ 2400 compounder, software-version 1.10 (Baxter Healthcare SA, Zurich, Switzerland).

Storage conditions

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 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 five days at 2–8 °C/dark. Table 2 overviews the course of the study.

Sample preparation for HPLC analysis

Test solutions in plastic syringes: 1.0 mL single aliquots were transferred from three test solutions of each syringe type to HPLC screw top vials and analysed without further dilution. Samples were taken on day (d) 0, 7, 14, 21, 28, 58, and 90 for test solutions stored at 2–8 °C. Sampling was performed on hour (h) 0, 4, 8, 12, 24, and on d 2, 7, 14, 21, 28, 58, 90 for test solutions stored at 20–25 °C.

Bulk solution: 1.0 mL single aliquots were withdrawn from each bulk solution on h 0, 4, 8, 12, 24 and on d 2, 5 and transferred to HPLC-vials.

HPLC assay

For determination of HI concentrations we used a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) assay. Validation was performed according to the *ICH Harmonized Tripartite Guidelines for Validation of analytical procedures: Text and Methodology Q2 (R1)* [18]. Details of the assay and its validation were published recently in this journal [6].

Table 2: Consolidated overview of the study procedures.

Test solution	Storage conditions	Analyses
Huminsulin® Normal 1 I.U./mL, Actrapid® Penfill® 1 I.U./mL in Original-Perfusor® syringe 50 mL	90 days at 2–8 °C/ dark or 90 days at 20–25 °C/diffuse room light	Human insulin concentration (HPLC)
	28 days at 2–8 °C dark plus 24 h at 20–25 °C/diffuse room light	pH, visible/subvisible particles
Huminsulin® Normal 1 I.U./mL in BD® Perfusion syringe 50 mL	90 days at 2–8 °C/ dark or 90 days at 20–25 °C/diffuse room light	Human insulin concentration (HPLC)
	28 days at 2–8 °C dark plus 24 h at 20–25 °C/diffuse room light	pH, visible/subvisible particles
Huminsulin® Normal 1 I.U./mL in ExactaMix® EVA (ethylene-vinyl acetate) 3 L bag	Five days at 2–8 °C/ dark	Human insulin concentration (HPLC)
Huminsulin® Normal 1 I.U./mL in B. Braun Ecobag® click (polyolefin) 3 L bag		

pH and visible/subvisible particles

Measurement of pH-values and visible/subvisible particle counts were performed as described in Ref. [6].

Results

Validation of the HPLC-assay

The correlation coefficient attained by plotting the obtained peak areas against the corresponding concentrations amounted to R^2=0.999 and proved linearity over the defined concentration range. The equations of the calibration curves were y=16,55,193x – 9,274 for Huminsulin® Normal 100 and y=21,35,072x – 12,968 for Actrapid® Penfill®. HI peaks of both brand products were observed at the retention time (RT) of about 10 min. A small peak at RT 3 min was attributed to m-cresol, an exicipient in both products.

The intra-day precision tests revealed a mean HI concentration of 1.022 I.U./mL ± 1.46% RSD for Huminsulin® Normal 100 and 1.004 I.U./mL ± 0.30% RSD for Actrapid® Penfill®. The inter-day precision tests revealed a mean HI concentration of 1.013 I.U./mL ± 1.02% RSD for Huminsulin® Normal 100 and 0.987 I.U./mL ± 0.41% RSD for Actrapid® Penfill®. The accuracy was –1.29% for Huminsulin® Normal 100 and 1.30% for Actrapid® Penfill®. The results met the criteria based on ICH Q2 (R1) and proved reproducibility [compare 6].

Concentration of HI injection solutions 1 I.U./mL

The HPLC chromatograms showed no peaks of impurities and degradation products over the whole observation period (Figures 1 and 2). Detailed results of the measured HI concentrations prepared in 50 mL disposable syringes are given in Tables 3 and 4. When Original-Perfusor® syringes were used as primary containers and stored refrigerated, HI concentrations added up to >95% after 21 days and >90% after 90 days, independent form the brand product used as starting material. A slightly higher rate of degradation was recognized when BD® Perfusion syringes were used as primary containers (compare Table 3). Storage at 20–25 °C

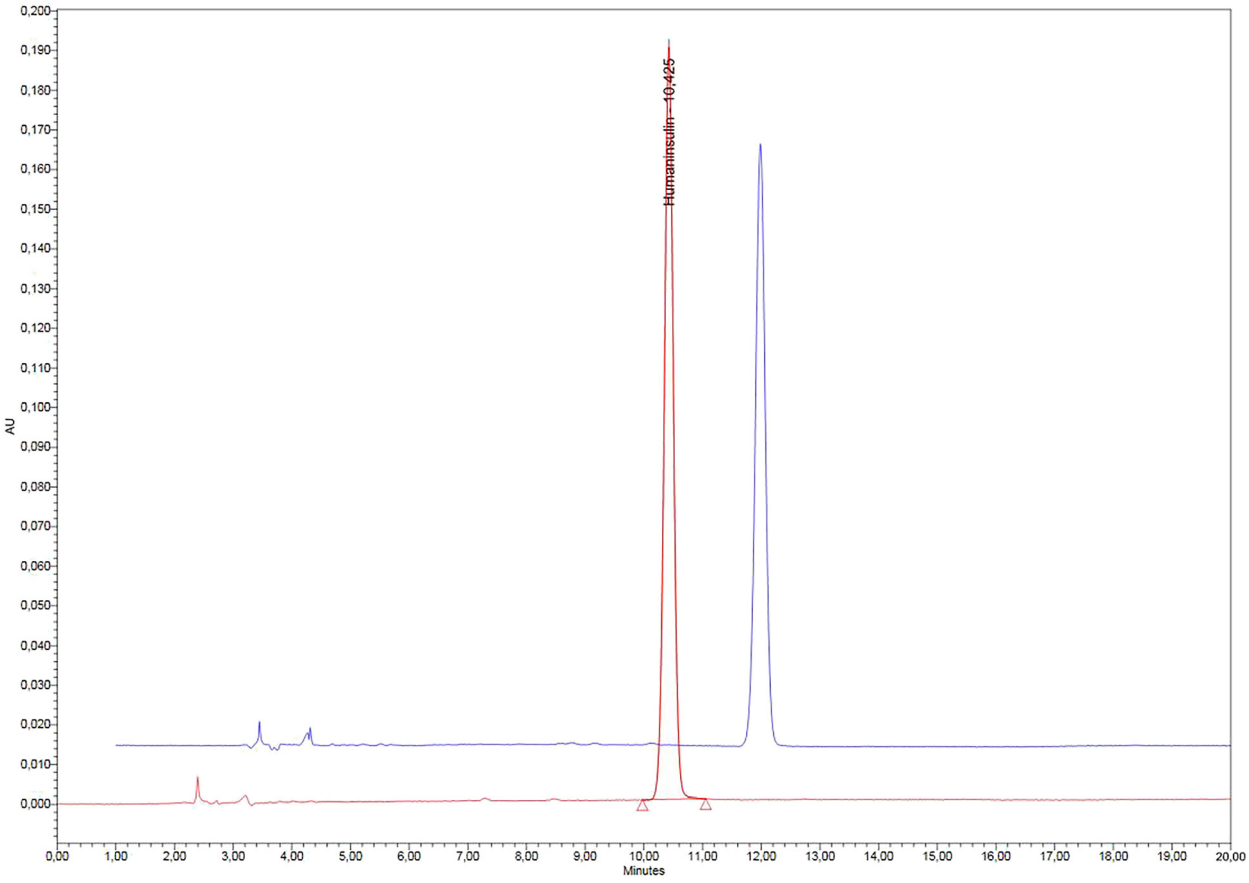

Figure 1: Representative HPLC-chromatogram of Actrapid® Penfill® 1 I.U./mL in Original-Perfusor® syringes, 50 mL. Red line d 0, blue line after 90 d of storage at 2–8 °C/dark.

without light protection excelerated the degradation rates. HI concentrations in the Original-Perfusor® syringes and in the BD® Perfusion syringes amounted to approximately 90 and 88% of the initial concentrations already at day 28, respectively (compare Table 4). After 90 days of storage at 20–25 °C, the HI concentrations fell to 82–84% of the initial concentrations. Indications for degradation products could not be detected in the HPLC chromatograms.

Depending on the material of the infusion bag used as primary container for preparation of the diluted bulk solution (Huminsulin®), the resulting HI concentrations differed considerably. When stored refrigerated in the dark for five days, HI concentrations decreased to 98.3 and 88.3% of the initial HI concentration in the polyolefin Ecobag® and the ExactaMix® EVA bag, respectively (Figure 3).

pH

Initial pH-values differed depending on the brand product (Huminsulin® 6.5, Actrapid® 6.3), whereas the syringe type hardly influenced pH. After 28 d of refrigerated storage and additional 24 h at room temperature, pH-values were in the range of 6.0–6.9 for Huminsulin® and at 6.5 for Actrapid® (Table 5).

Visible/subvisible particles

No colour changes or any occurrence of visible particles were observed during the whole observation period of 90 days. The results of subvisible particle counting are presented in Table 5. All test solutions met the requirements of the Ph. Eur. specification before and after the one month storage interval, i.e. maximum 6,000 particles ≥10 µm and maximum 600 particles ≥25 µm per container [19].

Discussion

The provision of diluted 1 I.U./mL HI-RTA-products by the pharmacy department is recommended to minimize risks

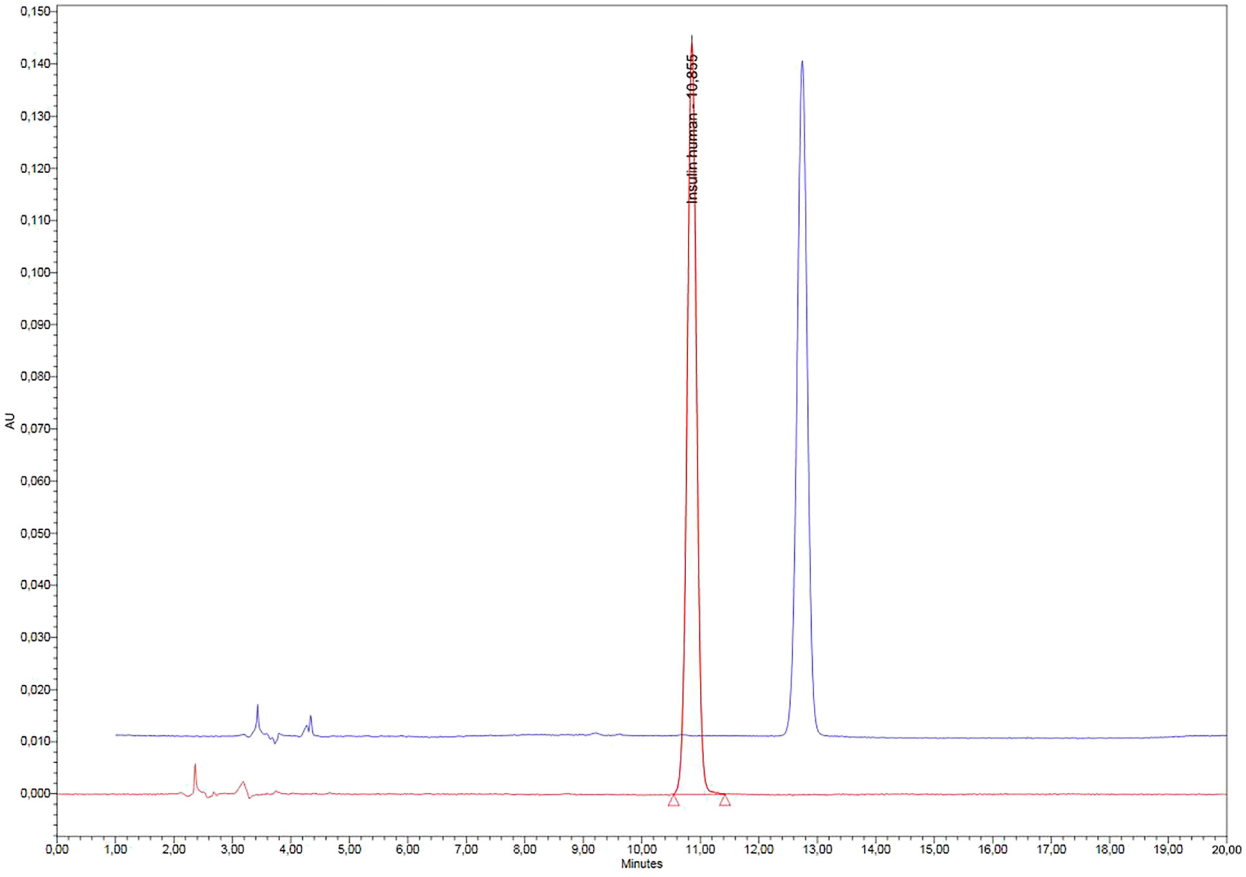

Figure 2: Representative HPLC-chromatogram of Huminsulin® Normal 1 I.U./mL in Original-Perfusor® syringes, 50 mL. Red line d 0, blue line after 90 days of storage at 2–8 °C/dark.

with reconstitution of the HI injection solutions [1, 20]. In hospital pharmacies batches of HI 1 I.U./mL injection solutions in plastic syringes can be prepared in series under identical aseptic conditions or aliquoted from the same initial bulk solution. The latter method is more efficient for bigger numbers of syringes. Anyway, shelf lifes of the preparations must be assigned by taking physicochemical and microbiological stability data into consideration [21]. Hence, we performed tests with different HI products available in Germany after dilution with 0.9% sodium

Table 3: Stability of human insulin 1 I.U./mL in Original-Perfusor® syringes or BD® Perfusion syringes, **kept at 2–8 °C/dark** for 90 days. Insulin concentration is given as percentage rate ± relative standard deviation (RSD) [n=9: three test solutions, one sample each, three injections of single samples].

	Syringe type	Initial human insulin concentration [I.U./mL] ± RSD		Percentage rate of the initial human insulin concentration [% ± RSD] Concentration 0 h=100%					
		Nominal	Measured	7 d	14 d	21 d	28 d	58 d	90 d
Huminsulin® Normal 100, Lilly	Original-Perfusor® syringe	1.00	1.011 ± 0.07	98.3 ± 0.2	97.6 ± 0.2	96.4 ± 0.0	95.8 ± 0.2	95.0 ± 0.6	93.9 ± 0.5
	BD® Perfusion syringe		0.999 ± 0.33	98.5 ± 0.6	97.7 ± 0.3	95.5 ± 0.3	94.4 ± 0.3	92.7 ± 0.4	90.3 ± 0.3
Actrapid® Penfill®, Novartis	Original-Perfusor® syringe	1.00	1.031 ± 0.56	96.5 ± 0.3	95.7 ± 0.7	95.0 ± 1.1	93.9 ± 0.6	93.7 ± 0.7	92.4 ± 0.6

Table 4: Stability of human insulin 1 I.U./mL in Original-Perfusor® syringes or BD® Perfusion syringes, **kept at 20–25 °C/diffuse room light** for 90 days. Insulin concentration is given as percentage rate ± relative standard deviation (RSD) [n=9: three test solutions, one sample each, three injections of single samples].

	Syringe type	Initial human insulin concentration [I.U./mL] ± RSD		Percentage rate of initial human insulin concentration [% ± RSD] Concentration 0 h=100%								
		Nominal	Measured	4 h	8 h	12 h	24 h	48 h	7 d	14 d	21 d	28 d	58 d	90 d
Huminsulin® Normal 100, Lilly	Original-Perfusor® syringe	1.00	1.013 ± 0.22	99.1 ± 0.2	98.9 ± 0.3	98.7 ± 0.4	98.1 ± 0.1	95.2 ± 0.1	95.2 ± 0.4	92.5 ± 0.3	91.6 ± 0.3	92.3 ± 0.3	86.3 ± 0.4	83.7 ± 1.3
	BD® Perfusion syringe		1.007 ± 0.28	99.2 ± 0.4	98.6 ± 0.2	98.6 ± 0.2	97.6 ± 0.1	96.9 ± 0.2	92.6 ± 0.3	91.9 ± 0.4	92.0 ± 0.4	87.8 ± 0.2	84.3 ± 0.9	82.0 ± 0.6
Actrapid® Penfill®, Novartis	Original-Perfusor® syringe	1.00	1.010 ± 0.59	99.7 ± 0.6	99.9 ± 0.7	99.9 ± 0.9	99.1 ± 0.6	99.2 ± 1.0	94.6 ± 0.5	92.9 ± 0.4	91.2 ± 0.6	90.2 ± 0.5	86.7 ± 0.4	83.6 ± 0.5

chloride solution and storage in 50 mL Original-Perfusor® B.Braun or BD® Perfusion syringes. In clinical practice injection concentrates in 10 mL vials, but not cartridges like Actrapid® Penfill, are used as starting material. However, we investigated the stability of diluted Actrapid® Penfill in Original-Perfusor® B.Braun syringes for comparison to the published data of Actrapid® Penfill in BD PlastiPak™ syringes as primary containers [7]. For quantification of intact HI, a RP-HPLC assay was implemented according to the known methods of the pharmacopeia and further publications [7, 17, 22, 23].

Our study revealed that physicochemical stability of HI 1 I.U./mL is mainly determined by temperature conditions and minor by the type of syringe used as primary container. This result corresponds to our findings gained with Insuman® Rapid, which turned out to be physicochemical stable in both types of test syringes when stored refrigerated [6]. Although decrease of HI concentrations is known to be caused by chemical degradation reactions [8, 9], we could not detect any degradation products in the HPLC chromatograms. This is most probably due to concentrations below the detection limit [6, 23]. Slight differences in the degradation rates of the different brand products may be caused by different formulations of the injection concentrates and different initial pH values of the test solutions. Neutral pH values are known to favour stability of HI solutions [6, 8]. An increased risk of precipitation at pH ranges of 4.5–6.5 is reported for Insuman® Rapid injection solutions [5]. Our recent investigation on Insuman® Rapid 1 I.U./mL solutions showed favourable pH-values (mean pH 6.9), presumably owing to the phosphate buffer used in the formulation [6]. Although lower pH values resulted when the buffer-free formulations of Huminsulin® Normal 1 I.U./mL and Actrapid® Penfill 1 I.U./mL solutions were diluted (compare Table 5), no precipitations were observed in the test solutions over the whole study period.

HI concentrations in Original-Perfusor® syringes exceeded the concentrations measured in BD® Perfusion syringes as primary containers over the whole observation period. This could be due to different airtightness of the syringes or different types and amounts of lubricants applied to the inner surface of syringes during the manufacturing process. HI stability in syringes is reported to be impaired by lower molecular weight silicone lubricants [24]. Since 50 mL syringes are always siliconized, information about the type of silicone lubricants used for manufacturing would be of interest but was not provided by the producers of the tested syringes. When Fleury-Souverain et al. investigated the stability of Actrapid® 1 I.U./mL in 60 mL BD PlastiPak™ syringes, they stated stability for six months (>90% of the initial HI concentration) under refrigeration and more than two

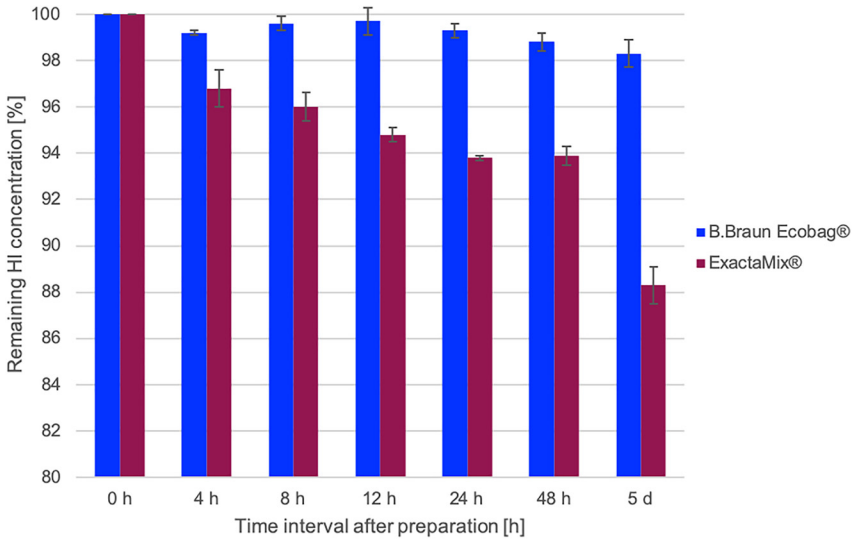

Figure 3: Stability of bulk solutions Huminsulin® Normal 1 I.U./mL in pre-filled 0.9% NaCl B. Braun Ecobag® and ExactaMix® EVA bag, **kept at 2–8 °C/dark** for five days. Insulin concentration is given as percentage rate ± relative standard deviation (RSD) of the initial concentration [n=3: one test solution, three injections of single samples].

months at room temperature [7]. The extended period of physicochemical stability could be related to the different type of syringes and lubricants used.

Numbers of subvisible particles in freshly prepared Huminsulin® Normal 100 test syringes were independent from the syringe type used. Actrapid® solutions showed less particles overall and the particle amount remained unchanged. Actrapid® is the only investigated HI containing zinc, which favours the formation of insulin hexamers, thereby preventing aggregation [9, 10]. A different picture got obvious after the four-week storage period with Huminsulin® Normal 100 solutions. Test solutions in Original-Perfusor® syringes showed decreased numbers of particles, compared to mostly unchanged numbers of particles in BD® Perfusion syringes. Possibly, the properties of air bubbles and silicone lubricants [24] are different in BD® Perfusion syringes, but this needs further investigation.

Pharmacy-based preparation of the RTA HI 1 I.U./mL in 50 mL syringes is normally performed by filling a bulk solution into empty syringes. Exemplary preparation and storage of Huminsulin® Normal 100 bulk solution 1 I.U./mL in two different infusion bags revealed that prefilled 3 L polyolefin bags (Ecobag® click B. Braun) are suitable containers. We tested the Ecobag® in comparison to an EVA bag, which has already been described to adsorb HI to a great extent [25, 26]. Yu et al. describe time and temperature dependent adsorption of Actrapid HM® 1 I.U./mL (in total 50 mL) filled into empty 500 mL EVA bags of approximately 60% after 48 h refrigerated storage [25]. In addition, Marcuard et al. do not recommend the use of EVA-bags for the

Table 5: Number of subvisible particles and pH in human insulin injection solutions 1 I.U./mL prepared in Original-Perfusor® syringes or BD® Perfusion syringes, stored for 28 days/dark at 2–8 °C plus 24 h at 20–25 °C/diffuse room light. Number of particles and pH values are given as mean ± relative standard deviation (RSD).

	Original-Perfusor® syringe, 50 mL				**50 mL BD® Perfusion syringe**			
	Three test syringes freshly prepared		**Seven test syringes after 29 d storage**		**Three test syringes freshly prepared**		**Seven test syringes after 29 d storage**	
	Number of particles ± RSD				**Number of particles ± RSD**			
	≥10 µm	**≥25 µm**	**≥10 µm**	**≥25 µm**	**≥10 µm**	**≥25 µm**	**≥10 µm**	**≥25 µm**
Huminsulin®, Normal 100, Lilly	2,377 ± 11	15 ± 42	105 ± 118	6 ± 62	3,113 ± 20	60 ± 11	3,498 ± 45	105 ± 67
Actrapid® Penfill®, Novartis	90 ± 37	13 ± 71	92 ± 45	9 ± 46				
	pH ± RSD						**pH ± RSD**	
Huminsulin® Normal 100 Lilly	6.46 ± 0.2		6.04 ± 1.3		6.44 ± 0.6		6.92 ± 1.7	
Actrapid® Penfill®, Novartis	6.26 ± 1.1		6.53 ± 1.6					

preparation of HI bulk solutions [26]. As adsorption is related to the surface area of the bag and the fraction of adsorbed insulin decreases with increased volumes of the same insulin concentration, the rate of adsorption was lower in our experiments, but also not acceptable.

Batchwise preparation of RTA HI 1 I.U./mL in the pharmacy-based central intravenous additives services requires microbiological stability testing. For the assignment of shelf lifes, results of integrity testing, microbial viability testing, media fills, and sterility tests performed during the validation phase of a product are to be considered. In our pharmacy, shelf lifes of aseptically prepared RTA products in disposable syringes are limited to a maximum period of three months according to the quality control and release criteria [27]. The same shelf life is applicable to HI 1 I.U./mL preparations regarding the proven physicochemical stability over 90 days when stored refrigerated.

Limitations of the study

Because of the destructive nature of the particle count test, additional test solutions had to be prepared and each test solution was usable for only one measurement. Of note, the testing was performed at other timepoints and intervals than the HPLC assays.

The stability indicating nature of the HPLC assay was not studied in detail for the licensed formulations Huminsulin® Normal and Actrapid® Penfill. The assumption was, that the active substance is identical to Insuman® Rapid, for which the RP-HPLC assay was implemented and successfully revalidated according to the tripartite guidelines Q2 (R1) [6]. Monographs of HI in the Ph.Eur. [22], USP [17], and further publications [7, 23] describe RP-HPLC methods with UV detection, suitable for quality control and stability tests of HI solutions. However, forced degradation studies with Huminsulin® Normal and Actrapid® Penfill using acidic and alkaline conditions were performed. The results proofed the stability indicating nature of the HPLC assay.

Conclusions

Ready-to-administer HI 1 I.U./mL solutions (Huminsulin® Normal 100, Actrapid® Penfill®) in 50 mL plastic syringes (Original-Perfusor® syringe or BD® Perfusion syringe) for continuous injection can be aseptically prepared batchwise in pharmacy-based central intravenous additive services and stored at 2–8 °C/dark for up to 90 days. Bulk solution (Huminsulin® Normal 100) can be prepared in polyolefin infusion bags prefilled with 0.9% NaCl solution, by addition of human insulin injection concentrates and filled into 50 mL plastic syringes as primary containers.

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Informed consent: Not applicable.
Ethical approval: The conducted research is not related to either human or animal use.

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