Physicochemical stability of human insulin 1 I.U./mL infusion solution in 50 mL polypropylene syringes

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Abstract

Objectives: The objective of this study was to investigate the physicochemical stability of human insulin 1 I.U./mL injection solutions (Insuman® Rapid) diluted with 0.9% NaCl solution in 50 mL disposable three-piece polypropylene syringes and stored refrigerated or at room temperature.

Methods: 1 I.U./mL test solutions were prepared with Insuman® Rapid and 0.9% sodium chloride infusion solution in 50 mL Original-Perfusor® syringes and BD® Perfusion syringes. Test solutions were stored for 90 days at 2–8 °C/dark or 48 h at 20–25 °C/diffuse room light in order to determine chemical stability. Additional test solutions were stored 28 days at 2–8 °C/dark followed by 24 h at 20–25 °C/diffuse room light to measure pH and particle counts. Human insulin concentrations were analysed by reversed-phase high-performance liquid chromatography at predefined time points. Test solutions were regularly inspected; subvisible particles and pH values were measured.

Results: Insuman® Rapid 1 I.U./mL injection solutions, stored at 2–8 °C/dark for 90 days showed a decrease of insulin content over time, regardless of the syringe type used. When kept at 20–25 °C/diffuse room light for 24 h at 20–25 °C/diffuse room light to measure pH and particle counts. Human insulin concentrations were analysed by reversed-phase high-performance liquid chromatography at predefined time points. Test solutions were regularly inspected; subvisible particles and pH values were measured.

Conclusions: Insuman® Rapid 1 I.U./mL injection solutions can be prepared by dilution with 0.9% NaCl infusion solution in disposable 50 mL three-piece polypropylene syringes as suitable primary containers. Physicochemical stability has been demonstrated for at least 21 days stored at 2–8 °C/dark followed by 48 h at 20–25 °C/diffuse room light.

Keywords: human insulin; intravenous injection; physicochemical stability; polypropylene syringe.

Introduction

In intensive care patients, continuous injections of diluted human insulin (HI) solutions are administered for the treatment of hyperglycaemic metabolic status. In general, standardized concentrations of diluted HI solutions are administered by syringe or infusion pumps and blood glucose levels are titrated by the injection speed and volume [1, 2].

Licensed medicinal products are used as starting material to reconstitute continuous injection solutions in the wards or to prepare ready-to-administer (RTA) injection solutions in pharmacy-based central intravenous additives services (CIVAS). In Germany, Insuman® Rapid 100 I.U./mL (Sanofi-Aventis Deutschland GmbH) is marketed as solution for injection in 10 mL multidose vials [3]. The marketed product contains, metacresol (Ph. Eur.), sodium dihydrogen phosphate-dihydrate, glycerol, sodium hydroxide, hydrochloric acid (for pH adjustment) and water for injection as excipients [3]. Type and volume of the vehicle solution to be used for the preparation of infusion solutions, as well as information on the compatibility and stability of diluted infusion solutions are not defined in the summary of product characteristics (SmPC) [3]. Moreover, to our knowledge studies concerning the physicochemical stability of diluted Insuman® Rapid infusion solutions have not been published yet.

HI consists of an A-chain (21 amino acids) containing an intramolecular disulfide bond and a B-chain (30 amino acids) connected by two interchain disulfide bonds. The chemical degradation is characterized by deamidation and the formation of covalent dimers and higher order polymers [4]. The chemical degradation is primarily influenced by pH, storage temperature and excipients used in the
formulation. Deamidation of A21 asparagine to aspartic acid represents the main degradation reaction in acidic solutions. At neutral pH, the deamidation reaction occurs very slowly at the B3 asparagine [4]. The physical stability of HI is mediated by noncovalent aggregation ending up in the formation of insulin fibrils and precipitates. Insulin containing medicinal products are formulated with m-cresol to preserve the formulation as well as to stabilize discrete insulin hexamers and to prevent aggregation. Changes in pH, exposure to elevated temperatures, agitation, and/or contact with hydrophobic surfaces can cause conformational changes that promote HI aggregation [4, 5]. In addition, insulin is associated with adsorption phenomena to various materials, e.g. glass, polyvinyl chloride (PVC), ethylene vinyl acetate (EVA), polyethylene (PE) and other polyolefine plastics [6–8]. Such materials are used for containers, tubings and filters of administration systems [8]. Factors influencing insulin adsorption include surface area, temperature, and the presence of proteins (e.g. human albumin), amino acids, glucose, and electrolytes [7].

The objective of this study was to determine the physicochemical stability of HI injection solutions 1 I.U./mL of Insuman® Rapid 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 48 h or 90 days.

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, Loughborough, UK; LOT: 1923632), acetonitrile for HPLC (Honeywell International Inc., Seelze, Germany; LOT: K0840); Insulin injection solution: Insuman® Rapid 100 I.U./mL, solution for injection in a vial, 10 mL (Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany; LOT: 9F043A, expiry date: 09/2021); Vehicle solutions: Pre-filled 0.9% sodium chloride 250 mL freeflex® infusion bags (Fresenius Kabi; LOT: 13PCF121, expiry date: 02/2023).

Primary packaging material and devices

Original-Perfusor® syringe 50 mL (B.Braun Melsungen AG, Melsungen, Germany; LOT: 20D05A8717); Injekt®-F 1 mL Luer Solo syringe (B.Braun Melsungen AG, Melsungen, Germany; LOT: 20E04C8), Microlance™ 3, 16 G × 1 ½” (BD, Becton, Dickinson and Company Limited, Louth, Ireland; LOT: 200410), screw top vials 1.5 mL, 32 × 11, 6 mm (ME-Analysentechnik GmbH, Mainz, Germany).

Preparation of human insulin 1 I.U./mL test solutions

Test solutions in polypropylene syringes: Each test solution was prepared under aseptic conditions by adding 0.5 mL Insuman® Rapid 100 I.U./mL concentrate to 49.5 mL 0.9% sodium chloride solution withdrawn from 250 mL freeflex® infusion bags. Thereby a nominal HI concentration of 1 I.U./mL was achieved. Diluted Insuman® Rapid test solutions were prepared in Original-Perfusor® syringes 50 mL as well as in 50 mL BD® Perfusion syringes as primary containers.

Storage conditions

Three Insuman® Rapid test solutions, prepared in the two types of 50 mL polypropylene syringes were stored for 90 days at 2–8 °C/dark or at 20–25 °C/diffuse room light for 48 h.

Another 10 Insuman® Rapid test solutions of each type were stored for 28 days/dark at 2–8 °C followed by 24 h at 20–25 °C/diffuse room light to measure pH-values and subvisible particles.

Sample preparation

Test solutions in polypropylene syringes: For HPLC analysis, 1.0 mL single aliquots were transferred from the three test solutions 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.

For pH measurement, approximately 4 mL single aliquots were withdrawn from the test syringes on d 0 and 29.

HPLC assay

HI concentrations were determined by a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) assay:

- Column: C18-Gemini®-column, 5 µm, 250 × 4, 6 mm, Art.-No. 00G-4435-E0, Organosilan material (TMS), Phenomenex Ltd. Aschaffenburg, Germany.
- Flowrate: 1 mL/min
- Injection volume: 20 µL
- Runtime: 20 min
- Detection wavelength: 214 nm
- Mobile Phase (pH 2.3): 34 parts phase A (2.9 mL phosphoric acid 85% ad 850 mL water HPLC grade), 34 parts phase B (17.9 g disodiumphosphate-monohydrate in 850 mL water HPLC grade), 32 parts phase C (acetonitrile for HPLC) / Flush solution: 95% Water for HPLC and 5% acetonitrile for HPLC [9].

The method is based on information provided by Sanofi-Aventis Deutschland GmbH [9]. The HPLC-System consisted of a Waters
Alliance 2.695 pump, connected to a Waters photodiode array detector 996 (Waters, Eschborn, Germany). Waters Empower Pro, Empower 2 Software, Version 6.10.01.00 (Waters Eschborn, Germany) was used for instrument operation, data collection and processing. Samples were injected in triplicate by an autosampler.

The injection solutions in syringes were declared chemically stable when the measured concentration was ±95% of the initial/nominal concentration designated as 100%.

**Validation of the HPLC assay**

Validation was performed according to the ICH Harmonized Tripartite Guidelines for Validation of analytical procedures: Text and Methodology Q2 (RI) [10]. Suitability of the HPLC method was tested by analyzing forced degraded samples of diluted Insuman® Rapid solutions. Insuman® Rapid solutions (1 I.U./mL) were either acidified with hydrochloric acid (1 M) or alkalinized with sodium hydroxide (1 M). After an exposure time of 60 min, the samples were assayed without prior neutralization.

For studying the linearity of the method, stock solutions were prepared by diluting 2.0 mL of Insuman® Rapid 100 I.U./mL with 48.0 mL water HPLC grade. Further dilutions with water HPLC grade were performed to achieve the following seven concentrations: 0.5, 0.8, 0.9, 1.0, 1.1, 1.2 and 1.5 I.U./mL. Aliquots of the calibration standards were injected in triplicate. The calibration curve was constructed by plotting the peak area vs. the nominal concentration of HI.

Intra- and inter-day accuracy and precision were tested by analyzing Insuman® Rapid solutions on five consecutive days. Standard solutions of the nominal concentration 1 I.U./mL, consisting of 1.0 mL of Insuman® Rapid in 100.0 mL water HPLC grade were freshly prepared. Ten test solutions were obtained by transferring 1 mL solution in 10 HPLC screw top vials. From test vial 1 and 10, samples were injected tenfold on five consecutive days. From test vials 2 to 9, a single injection was performed on the same days.

**pH measurement**

The pH-values were measured in triplicate in each sample with a Seven Compact Duo pH/Conductivity S213 pH meter (Mettler Toledo GmbH) and mean values calculated.

**Determination of visible and subvisible particles**

Whenever samples were withdrawn, each test solution was visually inspected for visible particles and colour changes.

Subvisible particles were counted with a calibrated PAMAS SVSS/SSBS (Partikelmess- und Analyse- systeme GmbH, Rutesheim, Germany [Serial-Nr.: 350-420]), equipped with a HCB-LD-50/50 sensor (Serial-Nr.: L-5050-5460) and the software USP V. 3.6.3. Prior to each test series, the sample port was flushed with water HPLC grade and after each test with 2-Propanol (Honeywell, LOT-Nr.: I1690). The aspiration tube of the apparatus was inserted into a test syringe and 10 mL samples of the test solution were automatically sucked in quadruplicate (~60 mL). Due to the required test volume, a different 50 mL test syringe had to be used for each counting. Three and seven test syringes of each type were tested at day 0 and day 29 (see storage conditions), respectively.

Each test series of particle counting consists of four consecutive tests 1–4, from which the result of test 1 is neglected. The mean number of particles measured in the tests 2–4 is automatically reported by the system and represents the mean number of particles in the test syringe.

According to Ph. Eur. 9.8, 2.9.19, solutions for injection in containers with a nominal content of less than 100 mL meet the specification of subvisible particles, when the number of particles ≥10 µm and ≥25 µm present in each container does not exceed 6,000 and 600, respectively [11]. Results were evaluated according to the Ph. Eur. specification [11].

**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 equation of the calibration curve was $y=181949x - 2833$.

HPLC chromatograms revealed the human insulin parent peak at a retention time of 9–11 min (compare Figure 1).

Under acidic and alkaline forced degradation conditions, the human insulin parent peak decreased 25 and 40%, respectively. Several small peaks of degradation products were detected, but none interfered with the insulin parent peak. The intra-day precision tests revealed a mean HI concentration of 0.997 I.U./mL ± 0.24% relative standard deviation (RSD), while the inter-day precision tests revealed a mean HI concentration of 0.998 I.U./mL ± 0.36% RSD. The results met the criteria based on ICH Q2 (RI) and proved reproducibility.

**Concentration of human insulin in injection solutions 1 I.U./mL**

Peaks of impurities and degradation products were not detected in the PDA chromatograms over the entire observation time (compare Figure 2). The chromatograms were characterized by sharp peaks and short retention times (about 9–11 min).

When stored refrigerated, stability of HI (>95% of initial/nomell HI concentration) was proved for 21 days with both syringe types. Detailed results of the measured HI concentrations are given in Table 1. Storage at 20–25 °C without light protection resulted in a slight decrease of HI in both syringe types (see Table 2). Accordingly, stability was proved for 48 h in Original-Perfusor® syringes and BD®
Perfusion syringes. Regardless of the storage conditions a slightly higher rate of degradation was recognized when BD® Perfusion syringes were used as primary containers.

pH

The pH-values of the diluted Insuman® Rapid solutions were measured at different time points and ranged between 6.6 and 6.9 in both syringe types (Table 3). Only at one sampling time point a pH value below 6.5 was measured in a single test solution, but returned to pH ≥ 6.5 at the following sampling time points.

Visible/subvisible particles

Visual inspection of the test solutions revealed no occurrence of visible particles and no colour changes up to the maximum storage periods. The results of subvisible particle counting are presented in Table 3. All test solutions met the requirements of the Ph. Eur. specification, i.e. maximum 6,000 particles ≥10 µm and maximum 600 particles ≥25 µm per container [11], before and after the 28 d refrigerated plus 24 h at 20–25 °C storage interval. In general, we could not detect an increase but rather a decrease of subvisible particles throughout the storage period.

Discussion

Intravenous administration of diluted HI 1 I.U./mL via continuous infusion/injection is common clinical practice for the treatment of hyperglycaemic metabolic status. The injection solutions are obtained by dilution of licensed HI concentrates filled in 10 mL vials, e.g. Insuman® Rapid 100 I.E./mL. Of note, information about suitable vehicle solutions and primary containers is not given in the relevant SmPC. To minimize the risks with reconstitution of the diluted HI injection solutions in clinical areas, the provision of ready-to-administer (RTA) HI-products is recommended [1, 12]. In this regard, batches of HI 1 I.U./mL

Figure 1: Representative HPLC-chromatogram of human insulin (retention time 9.985 min, 0.178 absorption units (AU); concentration 0.993 I.U./mL).
Figure 2: Representative PDA-chromatogram of Insuman® Rapid 1 I.U./mL in Original-Perfusor® syringes, 50 mL, d 90, stored at 2–8 °C/dark, Z-axis = nm.

Table 1: Stability of Insuman® Rapid 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=9:3 test solutions, three injections of single samples].

| Storage at 2–8 °C/dark | 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 |
| Original-perfusor®      |             | 1.00    | 1.051 ± 0.10 | 98.1 ± 0.2 | 97.7 ± 0.7 | 96.8 ± 0.2 | 96.3 ± 0.1 | 94.5 ± 0.3 | 93.3 ± 0.3 |
| BD® Perfusion syringe   |             | 1.041 ± 0.30 | 97.5 ± 0.2 | 96.6 ± 0.2 | 95.5 ± 0.4 | 94.7 ± 0.7 | 91.7 ± 0.6 | 90.6 ± 0.1 |

Table 2: Stability of Insuman® Rapid 1 I.U./mL in Original-Perfusor® syringes or BD® Perfusion syringes, kept at 20–25 °C/diffuse room light for 48 h. Insulin concentration is given as percentage rate ± relative standard deviation (RSD) [n=9:3 test solutions, three injections of single samples].

| Storage at 20–25 °C/diffuse room light | 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 | 4 h | 8 h | 12 h | 24 h | 48 h |
| Original-perfusor® syringe            |             | 1.00    | 1.022 ± 0.48 | 99.3 ± 0.4 | 99.4 ± 0.3 | 98.0 ± 0.5 | 98.6 ± 0.7 | 98.0 ± 0.5 |
| BD® perfusion syringe                  |             | 0.998 ± 0.20 | 98.9 ± 0.5 | 98.7 ± 0.2 | 98.6 ± 0.1 | 97.7 ± 0.6 | 97.2 ± 0.3 |
injection solutions in disposable syringes as primary containers can be prepared in series or aliquoted from the same initial bulk solution in hospital pharmacies under controlled aseptic conditions. In order to assign shelf lives to these pharmacy preparations, knowledge about physicochemical and microbiological stability is a prerequisite [13]. Hence, we determined the physicochemical compatibility and stability of Insuman® Rapid, after dilution with 0.9% sodium chloride solution and storage in 50 mL Original-Perfusor® B.Braun or BD® Perfusion syringes. The insulin concentration was determined by a RP-HPLC assay, based on information provided by the license holder of Insuman® Rapid [9] and successfully revalidated according to the tripartite guidelines Q2(R1) [10]. Similar RP-HPLC methods with UV detection are described in the Ph.Eur. and USP monographs of HI [14, 15] and further publications [6, 16].

The study results revealed that the physicochemical stability of Insuman® Rapid 1 I.U./mL is mainly determined by storage conditions and minor by the type of syringe used as primary container. Decrease of HI concentrations is most probably caused by chemical degradation reactions [4]. However, degradation products were not detected in the HPLC chromatograms due to concentrations below the detection limit (compare [16]). Further, degradation reactions are known to be pH dependent, and stability is favoured by neutral pH [4]. Favourable pH values were measured in Insuman® Rapid 1 I.U./mL solutions (mean pH 6.9), presumably owing to the phosphate buffer available in the concentrate for injection (pH 7.0–7.8) [3]. Dilution with 0.9% NaCl infusion solution (pH 6.0) resulted in a slightly decreased pH value, but generally pH values were higher than pH 6.5. This favours the solubility of HI, whereas a pH range of 4.5–6.5 increases the risk of precipitation [3].

Over the whole observation period, HI concentrations in Original-Perfusor® syringes exceeded the concentrations measured in BD® Perfusion syringes as primary container. 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. In this regard Nayef et al. [17] report that HI stability in syringes is impaired by lower molecular weight silicone lubricants. 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.

Comparable numbers of subvisible particles were counted in freshly prepared test syringes independent from syringe type. A different picture got obvious after the 4-week storage period. 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 [17] are different in BD® Perfusion syringes, but this needs further investigation.

Testing microbiological stability was outside the scope of this study. However, pharmacy-based batchwise preparation of RTA HI 1 I.U./mL requires microbiological stability testing. Results of integrity testing, microbiial viability testing, media fills, and sterility tests performed during the validation phase of a product are to be considered when shelf lives are assigned [13].

### Table 3: Number of subvisible particles and pH of Insuman® Rapid 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 28 d refrigerated storage plus 24 h at 20–25 °C |
| Seven test syringes after 28 d refrigerated storage plus 24 h at 20–25 °C | Three test syringes freshly prepared |

| Number of particles ± RSD per syringe | Number of particles ± RSD per syringe |
|--------------------------------------|--------------------------------------|
| ≥10 µm                               | ≥10 µm                               |
| ≥25 µm                               | ≥25 µm                               |
| ≥10 µm                               | ≥10 µm                               |
| ≥25 µm                               | ≥25 µm                               |
| 2105 ± 102                           | 1757 ± 63                            |
| 52 ± 96                              | 33 ± 23                              |
| 81 ± 52                              | 1066 ± 33                            |
| 6 ± 45                               | 44 ± 34                              |
| pH ± RSD                             | pH ± RSD                             |
| 6.86 ± 0.32                          | 6.68 ± 0.2                           |
| 6.58 ± 2.7                           | 6.92 ± 0.6                           |


Conclusions

Insuman® Rapid 1 I.U./mL injection solutions can be prepared by dilution with 0.9% NaCl infusion solution in disposable 50 mL three-piece polypropylene syringes as suitable primary containers. Physicochemical stability of Insuman® Rapid 1 I.U./mL injection solutions has been demonstrated for at least 21 days stored at 2–8 °C/dark and 48 h at 20–25 °C/diffuse room light.

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