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ORIGINAL ARTICLES

Compatibility of Insulin Lispro, Aspart, and Glulisine with the Solo™ MicroPump, a Novel Miniature Insulin Pump

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Abstract

Background:

This study compared the stability of commercially available, rapid-acting insulin in the novel tubeless, skin-adhering Solo™ insulin pump over 6 days at extreme environmental conditions.

Methods:

Forty-eight pumps for each tested analog were loaded with three different insulin lots and operated at 30 U/day (three sets of 12 pumps) and 15 U/day (one set of 12 pumps) with basal/bolus delivery patterns for 6 days under extreme climatic (37°C, 40% relative humidity) and mechanical (35 strokes/min) stresses. The insulin solutions dispensed were sampled periodically and analyzed for potency, related substances, high molecular weight proteins (HMWP), and preservative content by high-performance liquid chromatography techniques. Biological activity (bioidentity) was demonstrated by an abrupt decrease in blood glucose in rabbits. Solutions were inspected for visual appearance and measured for pH levels.

Results:

During the 6-day sampling period, the potency of all insulin samples was maintained at 95.0–105.0% of the bulk solution concentration of the insulin vials. The levels of HMWP and related substances remained well below labeling limits. The preservative concentration decreased with time but remained bacteriostatic effective. Solutions maintained pH and clarity and were particulate free. The biological activity was verified.

Conclusions:

Insulin analogs lispro, aspart, and glulisine maintained physical, chemical, and biological properties for 6 days when used in the Solo MicroPump device.

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Abbreviations: (BP) British Pharmacopoeia, (CSII) continuous subcutaneous insulin infusion, (HMWP) high molecular weight proteins, (SD) standard deviation, (USP) United States Pharmacopoeia

Keywords: Apidra, CSII, Humalog, insulin pump, MicroPump, Novolog, Solo

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Introduction

Diabetes mellitus patients require administration of varying amounts of insulin throughout the day to control their blood glucose levels. In recent years, ambulatory portable continuous subcutaneous insulin infusion (CSII) devices, known as "insulin pumps," have emerged as a superior alternative to multiple daily injections for type 1 diabetes patients.[1,2]

Insulin delivery via CSII devices is subjected to disruptive conditions such as mechanical stress, exposure to plastic and metallic compounds, light, and elevated temperature, leading to physical or chemical denaturation in the form of protein degradation, aggregation, fibrillation, or gelation.[3–7]

The stability of various rapid-acting insulin analogs was reported in currently existing "pager pumps," including the MiniMed 507c and 508 (Medtronic MiniMed, Sylmar, CA) and the Disetronic H-TRONplus (Disetronic Medical Systems, St. Paul, MN).[8,9] However, the compatibility of insulin analogs lispro, aspart, and glulisine with skin-adhered pumps has not been addressed to date.

The Solo™ MicroPump (Medingo Ltd) is a remotely controlled, tubeless pump intended for continuous subcutaneous delivery of insulin at set and variable rates. The current study was conducted to demonstrate the compatibility of the Solo MicroPump with three marketed rapid-acting insulin analogs under thermal and mechanical stresses at variable basal/bolus administration rates and a prolonged usage period of 6 days.

Methods

Materials and Equipment

The Solo MicroPump ("pump") is composed of a reusable pump base paired to a disposable insulin reservoir after filling (**Figure 1**). The device is detachable and is connected to a skin-adherable cradle after cannula insertion through a cradle opening. Each insulin analog was tested with 48 pumps and disposables (reservoir, cradle, and cannula).

Three commercial lots at different stages of insulin shelf life (within the expiration date) were obtained for each 100-U/ml rapid-acting insulin analog tested: Humalog® (insulin lispro, Eli Lilly), Novolog®/Novorapid® (insulin aspart, Novo Nordisk), and Apidra® (insulin glulisine, Sanofi-aventis).

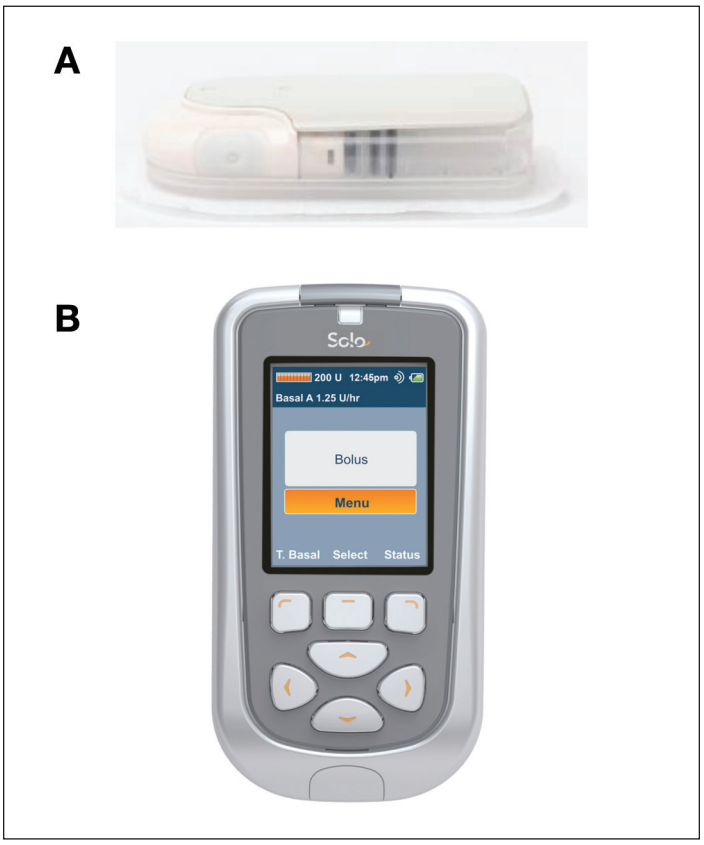

Figure 1. (A) A side view of the Solo™ MicroPump: pump base and disposable insulin reservoir are paired and connected to the cradle with adhesive. **(B)** Solo remote.

Procedure

The micropump reservoirs were filled with insulin, paired with a pump base, and connected to a cradle and cannula. Dispensed insulin was delivered to a vial via the cannula that pierced the vial rubber cork (**Figures 2A** and **2B**).

Filled pumps were secured to a multipump holder connected to a reciprocal shaker (FinePCR, Korea) inside the incubator (Binder KBWF-240, Germany) (**Figure 2C**). Pumps were wired to a personal computer for tracking real-time delivery rates, alerts, and alarms, simulating operation with the pump remote control.

Each sample was inspected visually with the Apollo II liquid viewer (Adelphy, USA). Daily samples obtained from the same test group were pooled in a single 4-ml vial for further analyses.

All experiments included 6-day pumping under controlled conditions of a temperature of 37 ± 2°C, 40% relative humidity, and continuous mechanical stress of 35 ± 5 strokes/min. Reference samples of insulin vial bulk solution (Control T = 0) and solution exposed to the same temperature and humidity but not to pumping (Control T = END) were analyzed for potency, preservative content, related substances, high molecular weight proteins (HMWP), and pH.

Delivery Rate at 30 U/day. Each insulin analog was tested in three test groups. In each test group, 12 pumps were loaded with one insulin solution batch and operated at a delivery rate of 0.6 U/h basal and three boluses of 5 units to simulate mealtimes (total of 29.4 units per day). Pooled collected samples from day 1 (0–24 hours), day 2 (24–48 hours), day 3 (48–72 hours), and day 6 (120–144 hours) were analyzed for potency, preservative content, related substances, HMWP, and pH. Pooled collected samples obtained at day 4 (72–96 hours) were used for potency and biological activity (bioidentity) tests.

Delivery Rate at 15 U/day. In a fourth test group, 12 pumps loaded with each insulin analog were operated at a basal rate of 0.3 U/h and three boluses of 2.5 units to simulate mealtimes (total of 14.7 units per day). A bolus of 30 units was delivered at the end of day 6. Samples obtained at days 1–2 (0–48 hours), days 5–6 (96–144 hours), and day 6 were inspected visually and analyzed for potency, preservative content, related substances, HMWP, and pH. Samples obtained at days 3–4 (48–96 hours) were analyzed for potency and bioidentity.

Analysis

High-performance liquid chromatography analytical methods for potency, related substances, and HMWP of insulin lispro and aspart were developed based on United States Pharmacopoeia (USP)[10] and British Pharmacopoeia[11] guidelines and were verified by Analyst Research Laboratories (Rehovot, Israel).

High-performance liquid chromatography analytical methods for testing potency, related substances, and HMWP of insulin glulisine were developed and validated by Almac Sciences (Craigavon, UK).

High-performance liquid chromatography analytical methods used to quantify the insulin solution preservative content (phenol and/or *m*-cresol) were developed and validated by Analyst Research Laboratories and Almac Sciences.

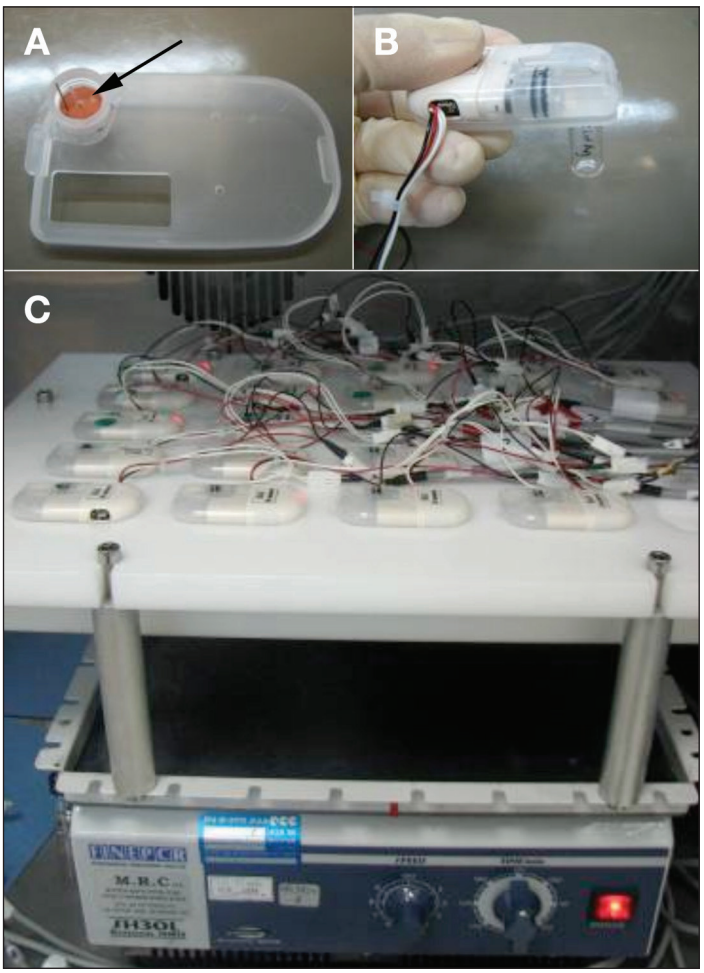

Figure 2. (A) Cradle and cannula assembly—the cannula (see arrow) is inserted through a 2-ml vial cork. A metal needle was inserted for pressure equalization. **(B)** Pump and collecting vial—wires were connected to a personal computer for real-time monitoring. **(C)** Multipump holder assembled on a shaker. Shaker and holder were placed in an incubator at 37°C, 40% relative humidity, and 35 rpm agitation.

The biological activity of insulin cannot be assessed by liquid chromatography methods. However, a qualitative test in rabbits can demonstrate the prominent manifestation of insulin activity. Bioidentity was assessed according to the USP guideline[12] by injecting a diluted portion of a tested and reference standard sample into eight rabbits and monitoring blood glucose levels at 0.5 and 2.5 hours following injection.

The visual appearance of all samples followed the European Pharmacopoeia guideline[13] using an Apollo II liquid viewer by inspecting solution clarity in front of either black or white panels. The pH of all solutions was measured with a pH meter.

Acceptance Criteria. Acceptance criteria were based on the referred pharmacopeia methods.[10–13] The limit

for bacteriostatic effectiveness was evaluated with five skin-related organisms at 28 days according to a USP monograph <51>[14] with lispro, aspart, and glulisine solutions having variable phenol and/or *m*-cresol concentrations. Preservative efficacy at 0.91 mg/ml (and above) *m*-cresol in lispro or glulisine and at 0.6 and 0.8 mg/ml (and above) of *m*-cresol and phenol, respectively, in aspart was demonstrated (data not shown).

A summary of testing methods and acceptance criteria for each tested parameter is shown in **Table 1**.

Results

Visual Appearance

All dispensed solutions were colorless, clear, and free from particulates.

Insulin Potency

Commercial vials contained rapid-acting insulin solutions at a concentration of 3.5 mg/ml or a potency of 100 U/ml. Insulin potency in dispensed solutions was presented in percentages relative to Control T = 0 sample (insulin vial bulk solutions).

The potency of all samples was within the 95.0–105.0% range (95–105 U/ml) (**Figure 3**). In the 30-U/day test, lispro potency showed higher variability within the three insulin batches [maximal standard deviation (SD) of 4.3% obtained on day 2] compared to glulisine (maximal SD of 1.0% on day 6) and aspart (maximal SD of 1.3% on day 1). Standard deviations of the "Control T = END" were 3.6, 0.3, and 0.2% for lispro, glulisine, and aspart, respectively, indicating varying sensitivity of lispro batches to 6 days of 37°C exposure.

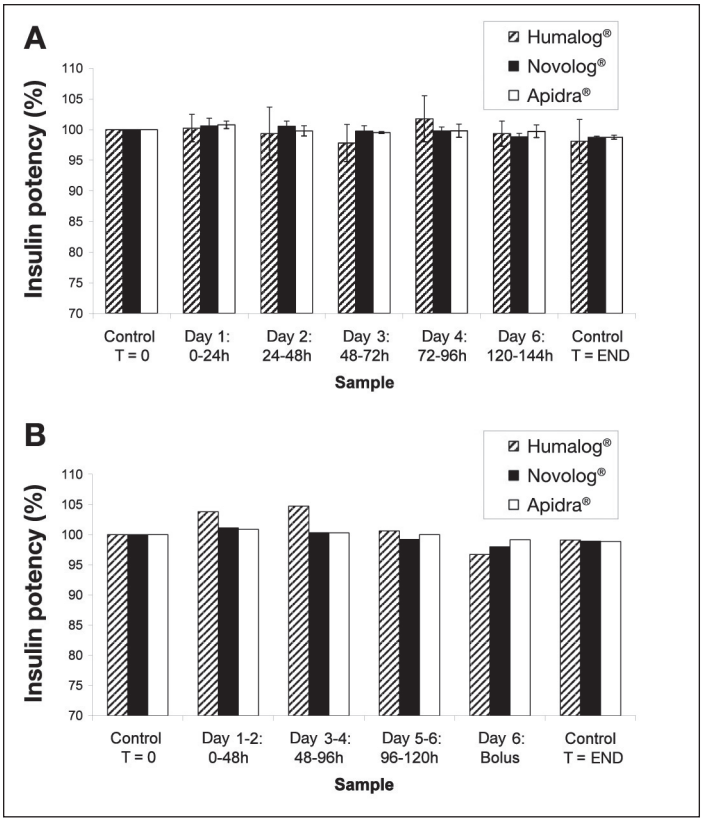

Figure 3. Insulin potency of Humalog®, Novolog®, and Apidra® at **(A)** 30-U/day test—results shown are the mean and SD of three lots—and at **(B)** 15-U/day test. Results are presented as relative percentages to Control T = 0 samples (bulk solutions of the insulin vial). "Control T = END" sample—insulin was exposed to thermal stresses but was not subjected to pumping action.

Preservatives

Insulin analog solutions contain antimicrobial preservatives: lispro and glulisine solutions–*m*-cresol (3.15 mg/ml), aspart solution–*m*-cresol (1.72 mg/ml), and phenol (1.5 mg/ml).

Table 1.
Test Properties and Their Accompanied Acceptance Criteria

Test	Apidra®	Novolog®/Novorapid®	Humalog®
Appearance	A colorless liquid, free from turbidity and foreign matter[13]		
HMWP	Not exceeding 1.50%[10,11]	Not exceeding 1.50%[11]	Not exceeding 1.50%[10]
Insulin assay	95.0–105.0%[10,11]	90.0–110.0%[11]	95.0–105.0%[10]
Preservative content	Preservative effectiveness[14] *m*-cresol >0.91 mg/ml	Preservative effectiveness[14] *m*-cresol >0.6 mg/ml phenol >0.8 mg/ml	Preservative effectiveness[14] *m*-cresol >0.91 mg/ml
pH	7.0–7.8[10,11,17]	7.0–7.8[11]	7.0–7.8[10,17]
Related substances	Total impurities <4.0%[10,11]	B28isoAsp <2.5% A21Asp + B3Asp + B3isoAsp <5% Other impurities <3.5%[11]	A-21 desamido <1.5% Other impurities <4.0%[10]
Bioidentity	>15 U/mg[12]		

In all samples, preservative levels were maintained well over the antimicrobial effectiveness threshold during the 6-day sampling period at 30-U/day (**Figure 4**) and 15-U/day (data not shown) tests. For the 15 U/day, minimal preservative content was obtained at days 5–6 (96–144 hours), resulting in lispro–1.76 mg/ml *m*-cresol, glulisine–1.71 mg/ml *m*-cresol, and aspart–1.04 mg/ml *m*-cresol, and 1.12 mg/ml phenol.

High Molecular Weight Proteins

High molecular weight protein values were maintained below the 1.50% threshold[10,11] during 6 days in all delivered samples at 30 U/day (**Figure 5A**) and 15 U/day (**Figure 5B**). Peak HMWP levels were 0.52, 0.34, and 1.10% for lispro, aspart, and glulisine, respectively. The HMWP level of the glulisine "Control T = END" sample was 1.07%, indicating a higher tendency to form HMWP entities in exposure to 6 days of 37°C.

Related Substances

Related substance (expressed in percentage) upper thresholds are described in **Table 1**. No criteria have been published for glulisine-related substances. Thus the upper limit was set similar to lispro at 4%.

Related substances were maintained below threshold during the 6-day test in all delivered samples at the 30-U/day test (**Figure 6**) and 15-U/day test (data not shown).

The A-21 desamido insulin lispro levels remained constant throughout the test period. The total level of other related substances increased with time, reaching a maximum of 2.02% on day 6 in the 30-U/day test (**Figure 6A**) and 1.57% on days 5–6 in the 15-U/day test. B28isoAsp insulin aspart, total A21Asp + B3Asp + B3isoAsp, and other related substances levels reached 0.88, 2.04, and 1.09%, respectively, on day 6 in the 30-U/day test (**Figure 6B**) and 0.69, 1.11, and 1.30% on days 5–6 in the 15-U/day test. The total A21Asp + B3Asp + B3isoAsp in Control T = END sample reached 2.06 and 1.03% in the 30- and 15-U/day tests, respectively, indicating that the increase is related to prolonged exposure to elevated temperatures. The peak total related substances of glulisine were 1.09% on day 6 in the 30-U/day test (**Figure 6C**) and 1.36% on days 5–6 in the 15-U/day test.

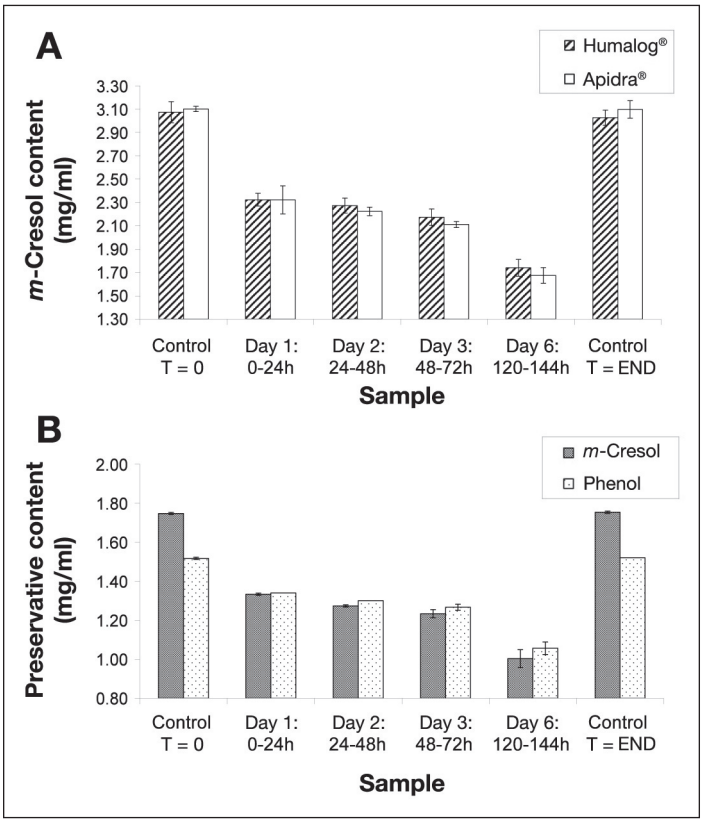

Figure 4. Preservative levels during a 30-U/day test of (A) *m*-cresol in insulin Humalog® and Apidra® and **(B)** *m*-cresol and phenol in insulin Novolog®/Novorapid®. Results shown are the mean and SD of three lots.

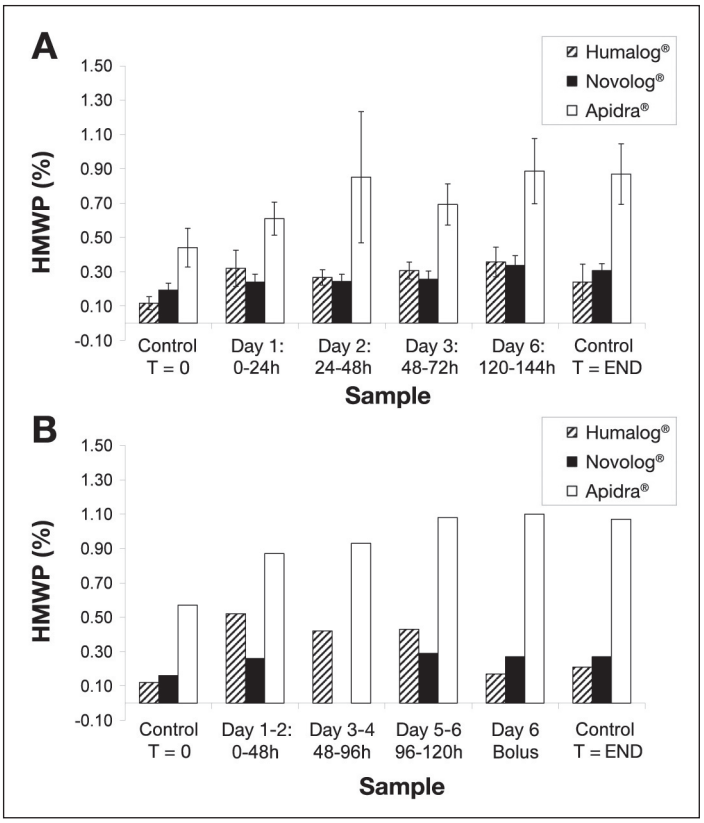

Figure 5. High molecular weight protein levels of Humalog®, Apidra®, and Novolog®/Novorapid® at **(A)** 30-U/day test—results shown are the mean and SD of 3 lots—and at **(B)** 15-U/day test.

pH

There were no significant changes in pH levels either in the 30-U/day test (**Figure 7**) or in the 15-U/day test (data not shown).

Insulin Biological Activity (Bioidentity)

The prominent biological effect of insulin is a decrease in blood glucose levels known as biological activity or bioidentity. A qualitative test in rabbits was conducted according to the bioidentity procedure described in USP monograph insulin assays.[12] All tested samples obtained calculated biological activity of at least 15 U/mg at both the 30-U/day test (37 ± 2.9 U/mg lispro, 44 ± 11.3 U/mg aspart, and 33 ± 3.5 U/mg glulisine) and the 15-U/day test (23 U/mg lispro, 40 U/mg aspart, and 22 U/mg glulisine).

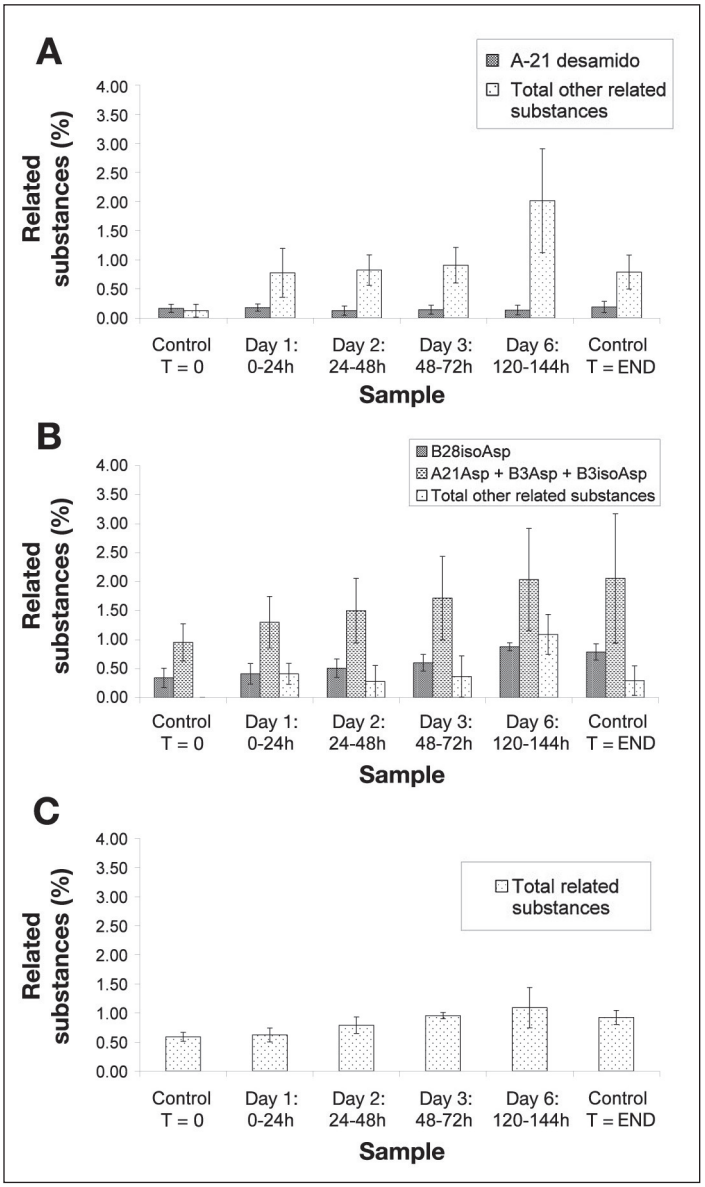

Figure 6. Related substance levels of **(A)** Humalog®, **(B)** Novolog®/Novorapid®, and **(C)** Apidra® at the 30-U/day test. Results shown are the mean and SD of three lots.

Discussion

Current insulin labeling is for 2 days of use in CSII. The Food and Drug Administration has approved Novo Nordisk Novolog for up to 6 days of use in CSII.[16]

The current study showed 6-day compatibility at extreme conditions of lispro, aspart, and glulisine with a novel skin-adherable Solo MicroPump (patch pump). These results are in agreement with previous studies that demonstrated the compatibility of insulin analogs lispro and aspart with "pager-like" CSII devices[8,9] for extended use periods beyond 2 days.

Some differences between a pager and a patch pump may affect insulin compatibility differently. In patch pumps, insulin may be exposed to a slight increase in temperature because of close proximity to the body surface. However, patch pumps are less prone to chemical, mechanical, and environmental stresses. For example, short tubing minimizes insulin contact with hydrophobic material, and firm skin adherence and mounting below clothing reduce shaking effects and exposure to direct light and extreme surrounding temperature fluctuations.

The Solo MicroPump was tested with three marketed rapid-acting insulin analogs under extreme thermal (37°C) and humidity (40%) conditions, which were well above skin temperature (32–33°C[17]) and below the relative humidity of skin.

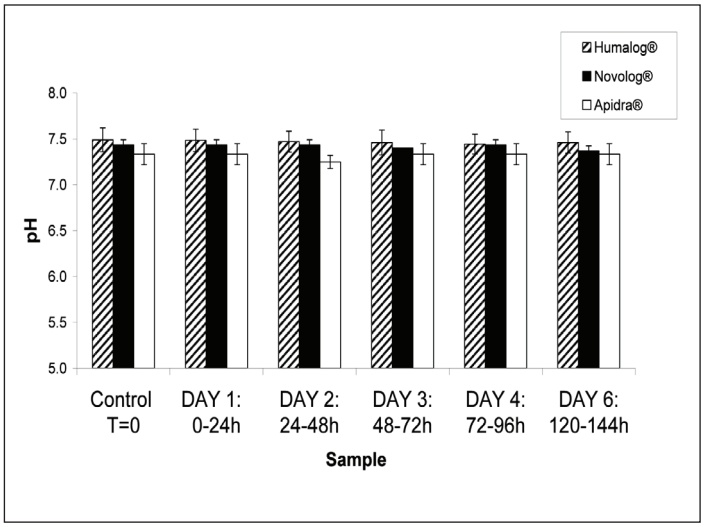

Figure 7. pH levels of Humalog®, Novolog®/Novorapid®, and Apidra® at the 30-U/day test. Results shown are the mean and SD of three lots.

The tested basal/bolus flow rates spanned typical diabetes treatment regimens and ranged from "average" consumption (30 U/day) to insulin-sensitive pump users (15 U/day) and insulin-resistant pump users (30 U bolus)

All tested insulin products maintained insulin potency for the 6-day period, indicating minimal solute adsorption or surface-induced denaturation.

Preservative content was decreased with time but remained well above the effective inhibitory level during the 6 days. A preservative decrease in "pager pumps" was greater in similar delivery patterns, resulting in 1.4–1.6 mg/ml *m*-cresol in Humalog during a 7-day study, as reported by DeFelippis and colleagues[9] and probably related to a diffusion of preservatives through plastics and polymers. It seems that in the current study, openings within the pump shell for personal computer wiring may enhance preservative loss. HMWP and related substance levels remained well below the upper threshold, even in extreme mechanical and climatic conditions. No particulates or visual change in insulin solution viscosity or transparency was detected during the 6-day observation period, reinforcing the lack of insulin physical degradation. Biological activity of dispensed insulin solutions in animals was confirmed, indicating that the biological characteristics of the insulin were maintained.

Conclusions

The current study demonstrated that the Solo MicroPump maintains the stability of Humalog, Apidra, and Novolog/ Novorapid for 6 days.

Acknowledgments:

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