Stability of U-500 regular insulin in prefilled syringes

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Stability of U-500 regular insulin in prefilled syringes

Abstract
Objective: To evaluate the stability of U-500 regular insulin in prefilled syringes stored under refrigeration for up to 28 days.

Methods: U-500 regular insulin was drawn up in 1 mL insulin syringes in a clean, nonsterile environment to emulate conditions of a patient’s home. Samples were assayed using a stability-indicating reverse-phase high-performance liquid chromatography method immediately after preparation (day 0) and after 7, 14, 21, and 28 days under refrigeration. Before evaluation, all samples were diluted to a concentration of 40 units/mL in the starting mobile phase. Stability was determined by evaluating the percentage of the initial concentration remaining at each time point.

Results: At least 93.3% of the initial U-500 insulin concentration remained throughout the 28-day study period, with no statistically significant changes in the amount remaining. The percent of initial concentration remained above 97% for the first 21 days of the study.

Conclusion: A prefilled syringe with U-500 regular insulin is stable for at least 28 days when stored under refrigeration. These data are similar to those reported for U-100 regular insulin, indicating that prefilling syringes with U-500 insulin is a safe and effective practice for patients who are unable to accurately draw up their own point-of-care doses.

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Keywords: U-500 insulin, prefilled syringes, insulin stability.
especially those in the community setting, be able to confidently counsel their patients on the ability to safely prefill their insulin syringes with U-500 insulin. The question of maintained efficacy with U-500 insulin in a prefilled syringe is one that is occurring with growing frequency given the increased use of U-500 insulin for the management of diabetes.

The stability of U-100 insulin preparations in pre-filled syringes has been well studied. Tarr et al. determined that U-100 regular insulin remains stable for 28 days at room temperature and under refrigeration. Dunbar and Simon reported that U-100 regular insulin maintains immunologic and biologic potency for at least 2 weeks. Zell and Paone found that U-100 regular insulin is stable under refrigeration in polypropylene syringes for at least 14 days. The previous two studies did not examine stability past 14 days. Unfortunately, no data currently exist regarding the duration of stability of U-500 regular insulin when stored in prefilled syringes. Because the stability of different concentrations of protein solutions can vary over time, data regarding the stability of U-500 may differ from that of U-100; therefore, an investigation of U-500 insulin is warranted.

Objective
The objective of this study was to determine if U-500 insulin is stable when stored in prefilled syringes at 4°C for up to 28 days.

Methods
Preparation of samples
The stability U-500 insulin (Humulin R U-500 Regular Insulin, Eli Lilly, Indianapolis, IN; lot A849585A, expiration February 2013) was evaluated after storage in 0.3 mL 29 gauge × 0.5 in polypropylene insulin syringes (BD Ultra-Fine Needle insulin syringe, Becton Dickinson, Franklin Lakes, NJ). Using careful technique on a clean but nonsterile laboratory bench, 0.25 mL (125 units) of U-500 insulin was drawn into each syringe (n = 3 for each time point). Three samples were analyzed immediately to establish baseline (day 0) stability values. The remaining 12 syringes were kept capped inside an open tray in a laboratory refrigerator (3–5°C). At days 7, 14, 21, and 28, three of the stored syringes were removed for analysis. Sample also was drawn for a forced degradation sample. In preparation for reverse-phase (RP) high-performance liquid chromatography (HPLC) analysis, each sample was diluted to an expected concentration of 40 units/mL in the starting mobile phase according to previously published protocols.

HPLC method
The RP-HPLC method previously described for use in U-100 stability studies was adapted for use with modifications according to standard insulin HPLC protocols used by the column manufacturer (Ascentis Express Peptide ES-C18 HPLC Columns, Sigma-Aldrich, St. Louis, MO). The HPLC instrument included a constant-flow solvent delivery system, variable volume injector, degasser, autosampler, and an ultraviolet light detector set to 214 nm (HPLC 2010A HT; Shimadzu Scientific Instruments, Marlborough, MA). The column used was a C18 column (4.6 × 30 mm) with a 500-Å pore size and 5-µm particle size (BioBasic C18 column; Thermo Fisher Scientific, Waltham, MA). Throughout the runs, the column was maintained at 40°C. Shimadzu LC Solution software (version 1.25; Shimadzu Scientific Instruments) was used for data collection and processing.

The mobile phase was a linear gradient of mobile phase A (deionized water and acetonitrile [90:10 v/v] with 0.1% trifluoroacetic acid) and mobile Phase B (deionized water and acetonitrile [25:75 v/v] with 0.1% trifluoroacetic acid). A linear gradient was used between two mobile phases, with a starting ratio of 75% A and 25% B, increasing to 40% B (60% A) over 15 minutes. The ratio then was programmed to increase to 60% B (40% A) from minute 15 to minute 20 to elute any remaining material from the column. A rinse then was performed with deionized water and methanol (1:1 v/v). Injections of 10 µL samples were eluted at a rate of 1.5 mL/min, with an average retention time of 4.8 ± 0.17 minutes. Each of the three replicates at each time point was run in duplicate on the HPLC. A standard curve of concentrations ranging from 0 to 100 units/mL was prepared to establish the linearity of the peak area versus concentration. The standard curve was linear (r² = 0.991) over the concentration range. The inter- and intraday variability of the RP-HPLC assay (as represented by coefficient of variation) were 0.23% and 0.05%, respectively.

Stability-indicating ability of the method was determined through the assay of a forced degradation sample. The forced degradation sample was prepared by adjusting the insulin to pH 2 with 10 N HCl and degrading the sample at 37°C for 96 hours. RP-HPLC analysis was conducted for this degraded sample in duplicate using the same method described above. The degraded sample showed decreased area of the main peak (52.3% remaining), and an unidentified degradation product peak was observed approximately 0.7 minutes after the insulin peak that is almost equal in area to the main insulin peak. No significant peak was observed at this time in other nondegraded samples.

Data analysis
The mean (±SD) area under the curve for insulin peak 1 was calculated. Mean values for days 7, 14, 21, and 28 were adjusted to a percent of the average day 0 value (measured at 500 units/mL; diluted sample measured at 40 units/mL). A one-way analysis of variance with a Bonferroni post hoc test then was performed to detect any statistically significant changes in insulin levels us-
Results
Under RP-HPLC, the area under the curve for peak 1 (retention time 4.8 ± 0.17 minutes) represents pharmaceutically active U-500. Samples at all measured time points retained at least 93.3% of the initial U-500 concentration (Table 1). Differences in percent of initial concentration remaining between any time point were not statistically significant (P = 0.130). This suggests a minimal loss of product due to degradation. Degradation products, not observed after 28 days of storage, can be seen in the forced degradation sample with a reduction in parent compound and the appearance of a second peak at 5.5 minutes.

Evidence in the literature is lacking regarding the definition of stability of insulin in the absence of statistically significant changes. In clinical practice, insulin adjustments often are made in increments of 10% to 20% of the given dose. Keeping this principal in mind, we considered stability to be maintained if at least 90% of the product remained at a given time point. Despite the fivefold increase in insulin concentration, it appears that U-500 insulin is at least as stable as U-100 insulin.4 At least 93% of the initial product remains when prefilled into syringes and refrigerated at 4°C with no statistically significant reductions in insulin concentration. A slight drop in percent remaining at day 7 is not statistically different from any of the other values and remains well above the 90% cutoff. The sample size gives adequate statistical power (>0.8); however, a larger sample size may help normalize the values. In addition, future studies should verify the sterility of U-500 insulin when drawn and stored in nonsterile conditions, though the sterility of U-100 insulin in prefilled syringes has been established.4

The prescribing information for U-500 insulin states that it must be used within 31 days of initial use or otherwise should be discarded.9 This should be taken into consideration when preparing prefilled syringes of U-500 insulin for subsequent storage. Additional consideration should be given to safely labeling and storing the syringes after they are prefilled because patients often have multiple doses of insulin. In practice, we recommend storing each dose in a different container, with each container in a different location in the refrigerator (e.g., prebreakfast dose on the top shelf, prelunch dose on the bottom shelf, predinner dose in the door). Caregivers also should be educated to label all insulin doses. We typically recommend large white mailing labels (approximately 2 in × 3 in). These labels can be attached to the barrel of the syringe with the number of units written in large print on one side and the meal written on the other. These measures are extremely important to minimize the risk of the patient injecting the wrong dose of their highly concentrated insulin and risking hypo- or hyperglycemia. It also is imperative that on the label, caregivers write in smaller print (for their use) the date that the U-500 vial was originally opened and the date the syringe was prefilled. These dates should be checked regularly to ensure prefilled syringes are used promptly and expired insulin is never administered by the patient.

Conclusion
U-500 insulin in prefilled syringes stored at 4°C is stable for up to 28 days. Prefilled syringes containing U-500 insulin and stored at 4°C for up to 28 days can be used confidently by caregivers for treating patients with diabetes with severe insulin resistance who are unable to independently manage a traditional vial and syringe.

References