5-2013

Stability of U-500 regular insulin in prefilled syringes

Melinda E. Lull
St. John Fisher College, mlull@sjfc.edu

Justin J. Piacentino
St. John Fisher College, jjp00470@sjfc.edu

Andrea N. Traina
St. John Fisher College

Publication Information

Lull, Melinda E.; Piacentino, Justin J.; and Traina, Andrea N. (2013). "Stability of U-500 regular insulin in prefilled syringes." *Journal of the American Pharmacists Association* 53.3, 304-306.

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.

Disciplines
Pharmacy and Pharmaceutical Sciences

Stability of U-500 regular insulin in prefilled syringes

Melinda E. Lull, Justin J. Piacentino, and Andrea N. Traina

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.

Keywords: U-500 insulin, prefilled syringes, insulin stability.

J Am Pharm Assoc. 2013;53:304–306.
doi: 10.1331/JAPhA.2013.12164

U-500 regular insulin is five times as concentrated as the more common U-100 regular insulin. U-500 can be ideal for patients requiring large volumes of insulin, such as those with immune responses to insulin, insulin receptor defects, or more commonly, severe insulin resistance secondary to obesity and long-standing diabetes. Clinicians are recognizing the benefits of using highly concentrated insulin, and use of U-500 insulin increased by 137% between June 2007 and June 2009.[1] Reducing the volume of solution injected also allows for more consistent absorption and improved glycemic control compared with use of large volumes of less concentrated insulin preparations.[2]

Currently, U-500 insulin is the only highly concentrated insulin product available in the United States. It also is the only insulin product that is not commercially available in a prefilled pen injection device. Some patients with diabetes may lack the vision or dexterity to accurately and safely draw up their own insulin doses using a vial and syringe.[3] This may be especially true of patients requiring U-500 insulin, as this may be indicative of more severe or progressive diabetes given their high degree of insulin resistance and large insulin dose requirements. The consequences of patient error in drawing up the correct dose can be much more grave when using concentrated insulin, as a 0.1-mL variation would be equivalent to a 50-unit change in dose rather than the 10-unit variation if a U-100 concentration insulin was used. To minimize the risk of dosing errors such as these, it is not uncommon for nurses, home health aides, or other caregivers to draw up several days of insulin syringes for a patient and leave them in a refrigerator for future use. It is imperative that pharmacists,

Received September 7, 2012, and in revised form December 6, 2012. Accepted for publication December 24, 2012.

Melinda E. Lull, PhD, is Assistant Professor of Pharmaceutical Sciences; and **Justin J. Piacentino, BS,** is a student pharmacist, Wegmans School of Pharmacy, St. John Fisher College, Rochester, NY. **Andrea N. Traina, PharmD, BCPS, BCACP,** is Assistant Professor of Pharmacy Practice, Wegmans School of Pharmacy, St. John Fisher College, Rochester, NY, and Clinical Pharmacy Specialist, Endocrine-Diabetes Care and Resource Center, Rochester, NY.

Correspondence: Melinda E. Lull, PhD, Wegmans School of Pharmacy, St. John Fisher College, 3690 East Ave., Rochester, NY 14618. Fax: 585-385-8453. E-mail: mlull@sjfc.edu

Disclosure: The authors declare no conflicts of interest or financial interests in any product or service mentioned in this article, including grants, employment, gifts, stock holdings, or honoraria.

Acknowledgments: To Dr. Fang Zhao (Wegmans School of Pharmacy, St. John Fisher College) for technical assistance with the high-performance liquid chromatography equipment and experimental design.

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 prefilled syringes has been well studied. Tarr et al.[4] determined that U-100 regular insulin remains stable for 28 days at room temperature and under refrigeration. Dunbar and Simon[5] reported that U-100 regular insulin maintains immunologic and biologic potency for at least 2 weeks. Zell and Paone[6] 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,[7] 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.[4]

HPLC method

The RP-HPLC method previously described for use in U-100 stability studies[4,8] 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 300-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 ($r^2 = 0.991$) 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-

ing SigmaPlot version 11.2 (Systat Software, San Jose, CA). $P < 0.05$ was considered statistically significant.

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.

Table 1. Stability of U-500 Insulin in syringes at 4°C

Day	Percent initial concentration[a] remaining Mean ± SD[b]
7	97.848 ± 0.629
14	99.432 ± 3.857
21	99.906 ± 2.389
28	93.317 ± 2.379

[a]Initial drug concentration = 500 units/mL.
[b]Mean ± SD of three biological replicates (n = 3).

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

1. Eli Lilly. IMS Health national prescription audit monthly, July 2005–July 2009. Indianapolis, IN: Eli Lilly; 2010.
2. Lane WS, Cochran EK, Jackson JA, et al. High-dose insulin therapy: is it time for U-500 insulin? Endocr Pract. 2009;15(1):71–9.
3. Fong DS, Aiello LP, Ferris FL 3rd, Klein R. Diabetic retinopathy. Diabetes Care. 2004;27(10):2540–53.
4. Tarr BD, Campbell RK, Workman TM. Stability and sterility of biosynthetic human insulin stored in plastic insulin syringes for 28 days. Am J Hosp Pharm. 1991;48(12):2631–4.
5. Dunbar JC, Simon FA. Immunologic and biologic evaluation of insulin degradation following prolonged storage in plastic syringes. Pancreas. 1986;1(4):317–9.
6. Zell M, Paone RP. Stability of insulin in plastic syringes. Am J Hosp Pharm. 1983;40(4):637–8.
7. Walsh G. Proteins: biochemistry and biotechnology. Chichester, UK: John Wiley & Sons; 2002.
8. Farid NA, Atkins LM, Becker GW, et al. Liquid chromatographic control of the identity, purity and "potency" of biomolecules used as drugs. J Pharm Biomed Anal. 1989;7(2):185–8.
9. Humulin R (Regular U-500 Insulin Human Injection, USP) [prescribing information]. Indianapolis, IN: Eli Lilly; 2011.