One-Year Thermostability of Commercial Glargine and Human Insulin

https://doi.org/10.2337/dc24-1749

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In a humanitarian crisis, in everyday life, and throughout the supply chain, insulin has the potential for inadvertent and undesirable exposure to conditions outside the recommended temperature range (2–8°C), raising concerns regarding its stability and efficacy. The U.S. Pharmacopeia (USP) established guidelines that stipulate insulin should possess a potency of 100 units/mL (±5 units/mL), and rigorous cold-chain supply systems are in place to maintain this temperature range for drug stability (1). To independently verify drug stability at the point of consumer purchase, we analyzed various formulations of insulin (i.e., human and analog; pens and vials; rapid, short, intermediate, and long acting; and premixed) acquired from retail pharmacies across five geographical regions in the U.S. and confirmed consistency with product labeling (2). Moreover, insulin purchased from the southeastern U.S. showed stability throughout various real-world exposures (i.e., shipment time, weather, and season), validating the efficacy of the traditional cold-chain supply (3). In resource-limited countries, where challenges in achieving the desired temperature range exist, alternative evaporative cooling approaches, such as storing insulin in clay pots, can extend product life (4,5). However, it remains critical to understand how long insulin retains its stability without access to recommended storage conditions. To address this knowledge void, we investigated the stability of unmodified (i.e., Humulin-R; Lilly) and the most commonly prescribed modified insulin (i.e., glargine U-100 [Lantus]; Sanofi) over a year under various experimental temperatures simulating hypothetical real-world settings.

Using the USP high-performance liquid chromatography with ultraviolet detection (HPLC-UV) techniques outlined for glargine and Humulin-R in USP monographs (www.uspnf.com) (2), we determined the concentration (insulin units per milliliter) for these insulins under five different temperature regimens at five time points over 1 year. The temperature conditions included steady 4°C, steady room temperature (RT) (22°C), steady 42°C, cycling from 4°C to RT daily (12-h intervals), and cycling from RT to 42°C daily (12-h intervals). Across all experimental conditions, insulin was quantified by HPLC-UV at baseline and 1, 3, 6, and 12 months thereafter. A different product vial was used for each of the 25 time point and temperature condition combinations to avoid bacterial contamination that repeated syringe punctures might cause, and all vials were from the same lot number. The UV and temperature data sets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

When stored as recommended (i.e., 4°C) and under 12-h cycling from 4°C to RT, both insulins maintained stability for at least one full year (Fig. 1). Both Humulin-R and glargine similarly maintained stabilities of 100 ± 5 U/mL for at least 6 months at RT, but at the 12-month measurement, both insulins fell below the range for USP acceptance. Interestingly, thermocycling (RT to 4°C) did not increase the degradation rate compared with corresponding steady-state temperatures. However, at 42°C both forms of insulin were subject to degradation, regardless of thermocycling. Glargine (Fig. 1*A*) showed better thermostability than Humulin-R (Fig. 1*B*), with both demonstrating an increased degradation rate with elevated storage temperatures.

The USP monograph is intended for use as a check for imperfect manufacturing formulations; therefore, the integrated area of the HPLC-UV peak is a sufficient measure for these analyses, as commercial formulations contain little to no degradation product. In contrast, the performance of time course thermostability studies, such as the one described here, could cause the formation of many interfering products. Hence, the 12-month time point for glargine under steady 42°C is likely an

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Received 19 August 2024 and accepted 9 September 2024

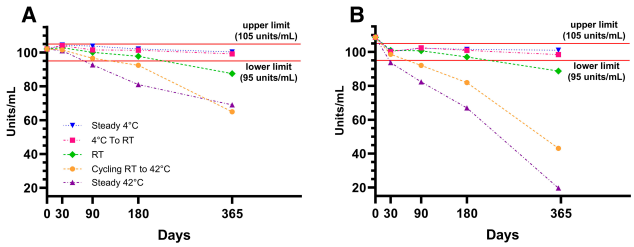

Figure 1—Insulin concentrations across 365 days. USP monographs were used for all analyses of glargine (Lantus) (*A*) and Humulin-R (*B*), with upper and lower limits of potency indicated with horizontal lines. Colors represent the temperature conditions.

overestimation of insulin concentration due to the observed incorporation of unresolved structurally altered insulin degradants in the quantification. Future long-term or high-throughput studies should consider developing new mass spectrometry methods for enhanced specificity and accuracy over UV detection that could be employed to test expired insulins, including those in undesirable settings of storage. Additionally, we believe these analyses of stability should be extended to other forms of insulin, including other glargine formulations as well as insulins increasingly used in pumps (e.g., lispro and aspart), and should employ in vivo methods designed to evaluate both insulin receptor binding and cellular activation.

These results raise the very pragmatic question of whether short-term inadvertent exposures to modest or high temperatures necessitate the immediate disposal of insulin. For example, while glargine was more thermostable than Humulin-R, both formulations remained highly stable under nearly all conditions tested for up to 1 month and even at RT for at least 6 months. These conclusions are presented based on assessments of insulin outside the recommended potency, yet it is significant to note that concentrations below this level still retained partial evidence of stability. These findings are particularly important for individuals with diabetes living in hot climates and/or for those with limited access to refrigeration. With emphasis, we are not calling for a cessation in the current cold-chain supply methods or recommended insulin storage conditions. Rather, we believe regulatory agencies should readdress their thermoregulatory storage guidelines with input from manufacturers and other professional diabetes organizations. In conclusion, we demonstrate that the resilience of insulin against thermal stress is greater than previously appreciated and has widespread economic and health care implications. When establishing real-world guidelines, pragmatic issues must be considered (e.g., travel refrigeration, storage outside of currently recommended temperatures, repeated puncture and use) while simultaneously considering efforts to reduce waste and lower costs of diabetes management as well as the growing impacts of climate change.

Acknowledgments. The authors thank various members from Breakthrough T1D, the American Diabetes Association, The Leona M. and Harry B. Helmsley Charitable Trust, and the USP for their long-term intellectual contributions to the performance of these studies.

Funding. This work was supported, in part, by funds from the University of Florida Diabetes Institute and The Jeffrey Keene Family Professorship.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. M.W.C. collected HPLC-UV data, analyzed the data, and wrote the manuscript. O.V., S.L., and S.M. researched the data, established the experimental conditions, and monitored and maintained insulin until the time of analysis. J.H.L. and S.F.B. collected HPLC-UV data. P.M.A. and M.J.H. provided oversight of the research and edited the manuscript. M.A.A. provided oversight of the study, acquired funds, and edited the manuscript. T.J.G. provided oversight of the research, reviewed all data, and edited the manuscript. T.J.G. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

M.J.H. and M.A.A. are editors of *Diabetes Care* but were not involved in any of the decisions regarding review of the manuscript or its acceptance.

Handling Editors. The journal editor responsible for overseeing the review of the manuscript was M. Sue Kirkman.

References

1. Devi S. Cold comfort: getting insulin to those who need it. Lancet 2021;398:1791
2. Garrett TJ, Atkinson P, Quinlivan EP, et al. Commercially available insulin products demonstrate stability throughout the cold supply chain across the U.S. Diabetes Care 2020;43:1360–1362
3. Garrett TJ, Feizbakhsh Bazargani S, Harmon T, et al. Commercially available insulin products demonstrate consistency with product labeling throughout all seasons in the U.S. Diabetes Care 2022;45:e166–e168
4. Pendsey S, James S, Garrett TJ, et al. Insulin thermostability in a real-world setting. Lancet Diabetes Endocrinol 2023;11:310–312
5. Richter B, Bongaerts B, Metzendorf M-I. Thermal stability and storage of human insulin. Cochrane Database Syst Rev 2023;11:CD015385