Thai Journal of Pharmacy Practice

Research article

A survey of Storage Temperature of In-use Human Insulins at Patients' Homes and Analysis of Their Stabilities under the Simulated Highest Temperature Identified in the Patients' Home

Korakoch Kangwantas[1], Sewan Theeramonkong[2,3], Natthabhum Kunathikom[1], Kaniknun Sansood[1], Juraporn Pongwecharak[1]

[1]Division of Pharmaceutical Care, Faculty of Pharmacy, Thammasat University (Rangsit Center)
[2]Division of Pharmaceutical Sciences, Faculty of Pharmacy, Thammasat University (Rangsit Center)
[3]Research Unit in Drug, Health Product Development and Application (DHP-DA), Thammasat University (Rangsit Center)

Abstract

Objectives: To obtain patients' household storage temperature of in-use human insulins, and to determine stability of human insulins under the simulated highest home storage temperature. **Methods:** Out-patients with diabetes (N = 47) who received either regular insulin (RI), isophane (NPH) or premixed RI/NPH insulins, used with a self-assembly pen were given a temperature logger to track storage temperature of in-use insulins for a consecutive 5-7 days. The highest and lowest storage temperatures were identified. Times per day the temperature being outside the recommended storage ranges were reported. Determination of the stability followed the analysis in the United State Pharmacopoeia (42nd ed.). Three batches of each insulin above were stored in temperature controlled cabinet under the isothermal highest home storage temperature identified previously. The assay for insulin content was performed at a weekly interval for 4 weeks, and was reported as percentage label amount, of which 95-105% was considered as passed. Physical changes were also observed. **Results**: Of the patients keeping insulin in a refrigerator (n = 22), 18 had temperatures outside the recommended 2 to 8°C range, with the average times amounted to 8 hours 30 minutes per day. The temperature was above 30°C for all patients reporting keeping insulin at room temperature, with the average times corresponding to 7 hours 57 minutes per day. The highest storage temperature identified was 43.6 °C. At the simulated isothermal 42±2°C, RI, NPH, and premixed RI/NPH insulins had insulin contents in the 95-105% range up to 2, 3, and 4 weeks, respectively. No significant physical changes were observed except an increase in viscosity of NPH and the premixed. **Conclusions:** Home storage temperatures of insulins were mostly out of the recommended ranges for about one-third of times in a day. Under isothermal temperature of 42±2 °C, RI, NPH, and RI/NPH insulins retained their acceptable potency at 2, 3, and 4 week, respectively.

Keywords: human insulin, storage temperature, household, diabetes, stability, refrigerators

Received: Feb 2, 2025, **Revised manuscript received:** Mar 21, 2025, **Accepted:** Mar 23, 2025

Correspondence: Juraporn Pongwecharak, Division of Pharmaceutical Care, Faculty of Pharmacy, Thammasat University (Rangsit Center), Pathumthani, 12121 **E-mail:** juraporn@tu.ac.th

PSU PRINCE OF SONGKLA UNIVERSITY
Thai Journal of Pharmacy Practice

บทความวิจัย

การสำรวจอุณหภูมิในการเก็บฮิวแมนอินซูลินที่เปิดใช้งานแล้วที่บ้านผู้ป่วยและการวิเคราะห์ความคงตัวภายใต้ภาวะจำลองของอุณหภูมิสูงสุดที่พบในบ้าน

กรกช กังวาลทัศน์ [1], ศรีวรรณ ธีระมั่นคง[2, 3], ณัฐภูมิ คุณาธิคม[1], กณิกนันต์ แสนสุด[1], จุราพร พงศ์เวชรักษ์[1]

[1]สาขาวิชาการบริบาลทางเภสัชกรรม คณะเภสัชศาสตร์ มหาวิทยาลัยธรรมศาสตร์ (ศูนย์รังสิต)
[2]สาขาวิชาวิทยาศาสตร์เภสัชกรรม คณะเภสัชศาสตร์ มหาวิทยาลัยธรรมศาสตร์ (ศูนย์รังสิต)
[3]หน่วยวิจัยด้านการพัฒนายา ผลิตภัณฑ์สุขภาพ และการนำไปใช้ มหาวิทยาลัยธรรมศาสตร์ (ศูนย์รังสิต)

บทคัดย่อ

วัตถุประสงค์: เพื่อเก็บข้อมูลอุณหภูมิบ้านที่ผู้ป่วยเก็บอินซูลินระหว่างใช้ และศึกษาความคงตัวของอินซูลิน ภายใต้ภาวะจำลองอุณหภูมิสูงสุดที่ได้จากข้อมูลอุณหภูมิบ้าน **วิธีการ:** ผู้ป่วยเบาหวาน (47 คน) ที่ได้รับอินซูลินชนิดใดชนิดหนึ่งต่อไปนี้ ชนิดออกฤทธิ์สั้น ออกฤทธิ์นานปานกลาง หรืออินซูลินผสมสำเร็จระหว่างชนิดออกฤทธิ์สั้นกับออกฤทธิ์นานปานกลาง ได้รับอุปกรณ์พกพาสำหรับติดตามอุณหภูมิเก็บอินซูลินระหว่างใช้เป็นเวลา 5-7 วันต่อต่อกัน การศึกษาเก็บข้อมูลอุณหภูมิสูงสุดและต่ำสุดที่พบในการเก็บยา ระยะเวลาต่อวันที่อุณหภูมิอยู่นอกช่วงที่แนะนำให้เก็บยา การศึกษาความคงตัวอ้างอิงวิธีการวิเคราะห์จากเภสัชตำรับสหรัฐอเมริกาฉบับตีพิมพ์ครั้งที่ 42 การศึกษาเก็บอินซูลินทั้ง 3 ชนิดอย่างละ 3 แบทช์ ในตู้ควบคุมอุณหภูมิโดยกำหนดอุณหภูมิเป็นค่าสูงสุดที่พบ การวิเคราะห์หาปริมาณอินซูลินทำทุกสัปดาห์เป็นเวลา 4 สัปดาห์ รายงานผลเป็นร้อยละของตัวยาสำคัญ โดยช่วงร้อยละ 95-105 เป็นช่วงที่ยอมรับได้ การศึกษายังสังเกตการเปลี่ยนแปลงลักษณะกายภาพของอินซูลิน **ผลการวิจัย:** ในกลุ่มผู้ป่วย 22 รายที่เก็บอินซูลินระหว่างใช้ในตู้เย็นพบว่า 18 รายมีอุณหภูมิการเก็บยาอยู่นอกช่วงที่แนะนำ (2-8 องศาเซลเซียส) ด้วยระยะเวลาเฉลี่ยประมาณ 8 ชั่วโมง 30 นาทีต่อวัน ผู้ป่วยที่เก็บอินซูลินระหว่างใช้ที่อุณหภูมิห้อง 25 ราย พบอุณหภูมิการเก็บที่สูงกว่า 30 องศาเซลเซียสทุกราย ด้วยระยะเวลาเฉลี่ยประมาณ 7 ชั่วโมง 57 นาทีต่อวัน อุณหภูมิสูงสุดคือ 43.6 องศาเซลเซียส ณ สภาวะจำลองอุณหภูมิสูงสุดที่ 42±2 องศาเซลเซียส อินซูลินออกฤทธิ์สั้น ชนิดออกฤทธิ์นานปานกลาง และชนิดผสม มีปริมาณอินซูลิน ในช่วงร้อยละ 95-105 ถึงสัปดาห์ที่ 2, 3 และ 4 ตามลำดับ ทั้งนี้ไม่พบว่ามีการเปลี่ยนแปลงทางกายภาพที่สำคัญ ยกเว้น อินซูลินชนิดออกฤทธิ์นานปานกลางและชนิดผสมสำเร็จมีความหนืดเพิ่มขึ้น **สรุป:** อุณหภูมิบ้านที่เก็บอินซูลินระหว่างใช้ ส่วนใหญ่อยู่นอกช่วงแนะนำ คิดเป็นระยะเวลาประมาณ 8 ชั่วโมงต่อวัน ภายใต้อุณหภูมิ 42±2 องศาเซลเซียส อินซูลินออกฤทธิ์สั้น อินซูลินออกฤทธิ์นานปานกลาง และอินซูลินผสมสำเร็จมีความคงตัวถึง 2, 3 และ 4 สัปดาห์ ตามลำดับ

คำสำคัญ: ฮิวแมนอินซูลิน อุณหภูมิเก็บ ครัวเรือน เบาหวาน ความคงตัว ตู้เย็น

Introduction

As a pharmaceutical product, insulins offer a flexible therapeutic option for people living with diabetes worldwide. Although they are no more considered earlier choices of antihyperglycaemic agent for type 2 diabetes mellitus, compared to several drug classes, insulin is more effective in glycemic control with no specific ceiling dose (1). In settings where cost is primarily a concern, human insulin formulations are alternatives for patients with type 2 diabetes (1). In Thailand human insulins have been included in the National List of Essential Medicines up to present (2). As it is a biological protein product, insulin is susceptible to degradation when exposed to light, heat and sheer conditions (3-4). High temperatures may also promote the formation of protein aggregates which could affect insulin quality (5).

In Thailand, available human insulins products are regular insulin (RI), isophane or Neutral Protamine Hagedorn (NPH) insulin, and premixed RI/NPH insulin, with different tradenames. A discrepancy in storage recommendations exists even for the same type (brand) of insulin that is marketed in different countries (6). Generally, it is recommended that unused human insulin products, regardless of brand, are refrigerated between 2 to 8°C. Once opened they can be stored at room temperature (15 to 30°C) and discarded after 28 days (7). However some Thai pharmacists advise keeping opened human insulins in the fridge because of concerns over possible insulin degradation if they are stored at ambient room temperature that exceeds 30°C. Even countries with a cold climate, storage temperature data of insulins kept in the fridge and carried insulins were reported to be out of the appropriate ranges, 100% and 50%, respectively (8). Over decades, the annual maximum temperature in Thailand had continuously risen with the annual mean maximum temperature of 33.7°C in 2020 (9). The number of days per year with temperature exceeding 35°C was 5 to 8 months, with a tendency for longer durations (10). Various cooling means were tried to offer an alternative choice in the setting with limited resources (11-12). The study in a sub-Saharan African country found almost 50% of patients with diabetes employed clay pots to keep in-use insulins cool (13).

In Thailand, little is known about patients' practice in storage of their insulin products and the actual storage temperature at home. One study showed 60% of their patients kept in-use insulins in the fridge (14). There is only one published study that tested insulin stability in simulated controlled temperatures (15). A study examining real storage temperature of insulin at patients' home had not been identified. The present study has 2 aims: 1) to obtain ambient storage temperature profiles of in-use insulins at patients' homes; and 2) to determine the stability of the insulin products under the simulated highest storage temperature identified in the patients' homes.

Methods

The study was approved, on October 30, 2019, by the local Human Research Ethic Committee (Science) Set 3, of the affiliated university (Project number 143/2562). Informed consent was obtained from all individual participants included in the study.

Study on storage temperature

Participant recruitment

Outpatients with diabetes were chosen from a tertiary hospital. The patients were purposively selected based on the following criteria: 1) 18 years of age or over; 2) receiving one of the following insulin products used with a self-assembly insulin pen, i.e., RI, NPH or premixed RI/NPH (30/70); 3) self-administered insulin; and 4) being literate. Availability of temperature data loggers (50 pieces) determined the number of the participants. At least 30 patients were considered to be sufficient to obtain consistent profile of the data. The recruitment process started from January 2020 to March 2020. Prescriptions of the insulins and the potential

participants were identified at the dispensing area of the Pharmacy Department. The process was independent from and did not intervene the routine care that the participants received from the hospital.

Determination of storage temperature

Each patient that consented to participate in the study was given one portable temperature data logger (Elitech® RC-5, USA). The devices were manufactured in the People Republic of China with the certificate of calibration. The participants were instructed to keep the temperature recorder with their insulin at all times. Temperatures were automatically tracked. The temperature recorders were collected after 5 to 7 days by appointment at participants' convenience. The data were transferred to a computer for analysis. Graphical temperature profile for each patient was retrieved. The data with less than 5 days of recording or showing the pattern which indicated switching between the fridge storage to outside the fridge or vice versa, were excluded from analysis.

Reported measurements

The proportion of patients whose insulin storage temperatures was beyond the recommended ranges (2-8°C for storage in refrigerator and > 30°C for storage in room temperature) was calculated for each group, i.e., in or outside refrigerator storage. The participants were asked to report where they placed their in-use insulins. The storage sites were then further confirmed with the temperature profile obtained from the temperature loggers. The highest (upper end) and lowest (lower end) temperatures were identified for the two groups. Proportions of the temperature data which were outside the recommended ranges were calculated on a 24-hour basis. The number of hours/day in which the temperatures were out of the recommended ranges was also estimated.

Stability study

Simulated temperature

The study assessed insulin stability at the simulated controlled temperature in a laboratory. The highest ambient storage temperature identified from the temperature profile at patients' home was used to represent the actual highest temperature out of the recommended range. Recommended storage temperatures (2-8°C for refrigerator and 25-30°C for room temperature) were simulated as control references. Three batches of the insulin products were stored separately at 3 different temperatures which represented the recommended refrigerator temperature (2 to 8 °C) [N = 7], the recommended room temperature (25 to 30°C) [N = 7], and the highest storage room temperature identified from the patients' temperature profile (42±2 °C) [N = 16].

Materials

Commercial RI (solution), NPH (suspension) and Premixed RI/NPH 30/70 (suspension) in cartridge containers (100 IU/mL) for use with a self-assembly insulin pen were studied. Lot numbers of the 3 batches of RI were JR70B94, JR71M14 and KR72B34. The corresponding numbers of NPH were JR70S38, JR71X20 and KR72V85. Those of premixed 30/70 were JR70S36, JR71W57 and KR73E60. Each type of the insulin products was of the same commercial name from the same manufacturer. The refrigerator was Sharp, SJP47N (Japan). The oven, Binder® model ED/FED (Germany) was used to set room temperature condition. The high temperature was set with the chamber, Binder® model KBF115 (Germany).

Human insulin USP reference standard (Lot number R032L0, content 26.5 USP human units/mg USA) was used. A 5-digit weighing balance (MS205DU) was used to weigh the standard human insulin. pH of the prepared samples was measured with a pH meter (model SevenCompact). A High Performance Liquid Chromatography (HPLC) instrument was the Shimadzu® system with a SPD-20A System Controller. Detailed appearance of samples were examined with a 4-fold magnifying glass and the 20x magnification microscope (Nikon Eclipse Ts2 with digital imaging model DS- Fi3 and NIS Elements D).

Insulin analysis

For HPLC conditions, the analyses were carried out on RP C18 column (Inersil® ODS-3 4.6 x 150 mm, 5 μm) as a stationary phase. The mobile phase was prepared as described in "Insulin Human Injection" of the United State Pharmacopeia (USP), 42nd edition (16), containing 26% acetonitrile and 74% aqueous anhydrous sodium sulfate (2.84%w/v) and 0.27 mL of phosphoric acid per 100 mL of mixture. pH of the mixture was adjusted to 2.3 with ethanolamine. This mixture was then filtered through 0.45 μm nylon membrane filter and degassed for 30 min in ultrasonic bath prior to use. The wavelength was 214 nm and the flow rate was 1 mL/min. Twenty microliters of sample or standard solution were injected. All analyses were carried out at 40 °C. The run time was 18 minutes. The reference standard was 40-unit Human insulin/mL in 0.01 N hydrochloric acid. The sample solution was prepared following the official protocol of the monograph of insulin injection.

The products stored at 42±2 °C were analyzed at days 0, 7, 14, 21 and 28. For the control group, the analyses were carried out at days 0, 21 and 28. All insulin samples were drawn out and discarded one unit each time to mimic daily insulin use.

Areas under curves (AUC) of chromatogram represented insulin content quantification, with a relative standard deviation of less than 1.6%. Calibration curves were obtained from 3 independent injections of 5 concentrations of insulin (24, 32, 40, 48 and 56 unit/mL.)

Physical study

The solutions of each sample were visually inspected for clarity every week. The pH was determined at day 0, 14 and 28, with reference to the USP acceptable range of 7.0 to 7.8 (16). The presence of precipitates was inspected at day 28 under visual observation and microscope.

Data analysis

Microsoft Excel 2019 and SPSS^{X} version 26 were used for data analysis. Descriptive statistics, i.e., frequency/percentage, median [interquartile range, IQR] and mean [standard deviation, SD] were reported for proportional data and temperature data, respectively. An independent t-test was used to compare percentage label amounts of insulin content at different storage temperatures, with a p-value < 0.05 indicating statistical significance.

Results

Forty-seven patients managed to return the temperature loggers. All reported sites of in-use insulin storage corresponded with the temperature profiles.

Ambient storage temperatures

Table 1 shows ambient storage temperature of in-use insulins at patients' home. Among 47 patients, 22 (48.6%) kept in-use insulins in a refrigerator. Four of 22 (18%) had their all-time temperatures in the recommended range (2 to 8°C). The average upper end temperature was 10.08[4.34]°C with median of 9.75 [4.8]°C. The corresponding figure for lower end temperature was 3.68[4.6] °C with median of 2.4[4.4] °C. Upper end temperature above 8 °C was recorded in 64% (14/22) of the patients. On average, 35.4% (510/1440) of temperature data measured in 24 hours were out of the recommended range, amounting to 8 hours 30 minutes per day.

Over 50% (25/47) of the patients stored in-use insulins outside the fridge (room temperature). All of 25 patients had their storage temperature out of the upper recommended range (30°C). The average upper end temperature was 34.4[2.6] °C with median of 34[2.7] and 43.6°C being the highest storage temperature recorded. The corresponding figure for lower end temperature was 23.3[1.2] and median of 23.3[2] °C. Thirty-three percent (477/1440) of room temperature data measured within 24 hours were out of the 25-30°C range, amounting to 7 hours 57 minutes per day.

Table 1. Summary of ambient storage temperature of in-use insulins at patients' home (N = 47)

Temperature	Storage place	
	Refrigerator	Outside refrigerator (room temperature)
N (%)	22 (48.6)	25 (51.4)
Upper end temperature, °C		
Mean (SD)	10.08(4.34)	34.4(2.6)
Median (IQR)	9.75(4.8)	34(2.7)
Min, Max	3, 20.7	31.5, 43.6*
Lower end temperature, °C		
Mean (SD)	3.68(4.6)	23.3(1.2)
Median (IQR)	2.4(4.4)	23.3(2)
Min, Max	(minus) 2.7, 16.5	21.4, 25

*Highest temperature identified

Insulin assay

Table 2 displays percentage label amount of three batches of RI, NPH and premixed RI/NPH insulins under 3 studied conditions. Under 42 ± 2°C, RI content decreased at day 21 and appeared to stabilize through day 28, with 2 batches showing marginal out of the acceptable 95-105% range at 21 days. Those of NPH and premixed RI/NPH insulins also dropped at day 21 but remained well in the acceptable range. By day 28, RI and NPH insulin lost potencies (i.e., less than 95% of insulin content), which conformed to the expiry date of opened products regardless of temperature (7). All batches of premixed RI/NPH insulin maintained acceptable content at day 28. (Table 2). All reductions in percentage label amount measured at baseline (day 0) and day 28 were significant ($P < 0.05$) (Fig. 1).

Physical study

Under 42 ± 2°C, no physical change was observed in all insulin samples throughout 28 days, except that NPH and premixed RI/NPH insulins appeared more viscous after 7 days. At day 28, all insulin samples showed no evidence of precipitates under visual inspection as well as microscope (not shown). The color of all samples had not changed. The pH of RI increased at days 21 and 28 (from 7.22 to 7.50 and 7.55, respectively) but was confined within the acceptable range (7.0 to 7.8). The pH of other insulins was relatively stable (7.2-7.5) (not shown).

Discussion

The present study was the first in Thailand to identify actual temperature in patients' home for insulin storage. It was evident that the patients with diabetes in the study differed in how they stored their in-use insulin pens. Almost 50% of the patients refrigerated their in-use insulins. The finding was consistent with 60% of out-patients in the other study in Thai university hospital (14). This may reflect discrepancy in pharmacists' advices as to the appropriate place to keep in-use insulin. Although it is not necessary for in-use human insulins to be kept in the fridge, in actual practice pharmacists might advise the patients to do so to safeguard against potential degradation. However, majority of refrigerator storage temperature data fell outside the appropriate 2-8 °C. The result was comparable to that reported by Braune et al (8). Lower end or subzero temperatures were identified in 36% (8/22) of the patients, compared to 25% in the study cited above (8). For subzero storage temperature,

insulin potency was certainly lost. The number of times where temperature data being outside 2-8°C was 3-fold higher compared to that of Braune et al (8). Variations of household refrigerator in terms of quality, function, and daily use load depend on several factors. The issue thus posts a challenge for healthcare professionals who provide a standard advice on keeping spare human insulin products in a refrigerator which is expected to be of 2-8°C. However the recent study in India demonstrated that without refrigerator unopened products of human insulins and some insulin analogs were thermostable up to 2-4 months under storage temperature of 25-35°C in real life setting (17).

For those with room temperature storage of in-use insulin, the ambient temperature was out of 30°C in all patients with the highest temperature recorded at 43.6°C. The average time out of the recommended

Table 2. Percentage label amount of three batches of RI, NPH and premixed RI/NPH insulins under controlled–temperature refrigerator (F), room temperature (RT) and high temperature (HT), measured at weekly interval

RI	Percentage label amount of insulin														
Day	F_1	F_2	F_3	F_{av}	SD	RT_1	RT_2	RT_3	RT_{av}	SD	HT_1	HT_2	HT_3	HT_{av}	SD
0	102.8	101.4	101.2	101.8	0.71	102.8	101.4	101.2	101.8	0.71	102.8	101.4	101.2	101.8	0.71
7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	98.8	98.9	99.3	99.0	0.22
14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	98.9	97.4	96.9	97.7	0.85
21	102.3	100.7	101.6	101.5	0.65	102.1	100.5	101.1	101.2	0.66	97.2	94.3	94.6	95.4	1.30
28	101.5	101.3	101.5	101.4	0.09	101.4	99.1	99.9	100.1	0.95	93.5	94.8	94.7	94.3	0.59
NPH	Percentage label amount of insulin														
Day	F_1	F_2	F_3	F_{av}	SD	RT_1	RT_2	RT_3	RT_{av}	SD	HT_1	HT_2	HT_3	HT_{av}	SD
0	102.0	100.8	101.9	101.6	0.54	102.0	100.8	101.9	101.6	0.54	102.0	100.8	101.9	101.6	0.54
7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	101.4	98.9	98.7	99.7	1.23
14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	101.2	97.4	98.9	99.2	1.56
21	101.6	100.7	101.1	101.1	0.37	101.9	99.6	101.2	100.9	0.96	99.1	96.5	98.9	98.2	1.18
28	101.8	100.4	101.7	101.3	0.64	101.7	99.3	98.2	99.7	1.46	93.7	93.6	94.0	93.8	0.17
RI/NPH	Percentage label amount of insulin														
Day	F_1	F_2	F_3	F_{av}	SD	RT_1	RT_2	RT_3	RT_{av}	SD	HT_1	HT_2	HT_3	HT_{av}	SD
0	102.6	101.3	102.7	102.2	0.64	102.6	101.3	102.7	102.2	0.64	102.6	101.3	102.7	102.2	0.64
7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	101.4	100.3	101.5	101.1	0.54
14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	101.0	100.7	100.3	100.7	0.29
21	102.5	101.7	102.0	102.1	0.33	102.9	100.7	101.9	101.8	0.90	100.5	100.8	100.2	100.5	0.25
28	102.3	101.6	102.2	102.1	0.31	101.6	100.8	101.7	101.4	0.40	97.9	97.0	95.5	96.8	0.99

Note: F, 2-8°C; RT, < 30°C; HT, 42±2°C

av, average value; SD, standard deviation; ND, not determined.

1, 2, and 3 represent batch number of insulins.

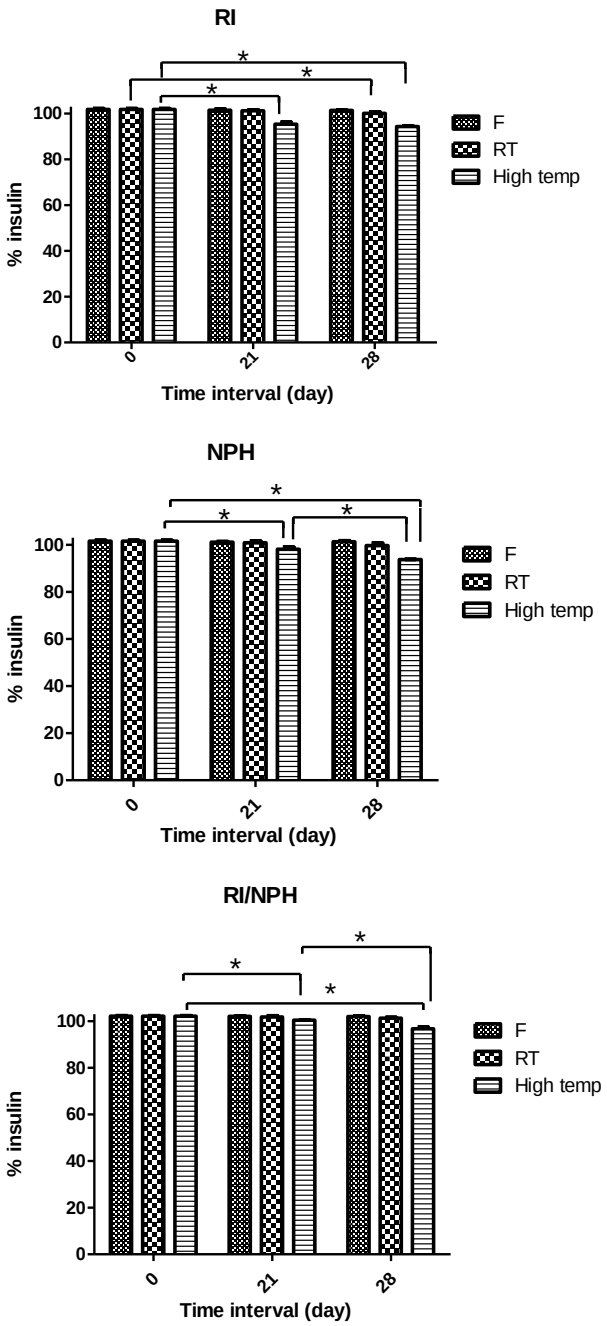

Fig. 1. Percentage label amounts of RI, NPH and premixed RI/NPH insulins, under controlled-temperature refrigerator (R, 2-8°C), controlled room temperature (RT, 25-30°C), and isothermal 42±2°C (High temp), measured at different intervals.

* denotes significant difference at $P < 0.05$

temperature at 25-30°C corresponded to an approximate 8 hours per day, i.e., one-third of a day. The result was in contrast to less than 10 minutes a day reported in the study done in western countries (8), reflecting possible less variation of temperature in the context of cold climate countries. In routine patient education, Thai pharmacists uniformly emphasize that the middle compartment of refrigerator is the most appropriate location to keep insulin either spare or opened, because its cooling temperature is assumed to be constant and in the acceptable range. Sites to be avoided are the refrigerator door, freezing chamber and the compartment immediately adjacent. The reasons are probable fluctuations of temperature when the door is opened each time as well as the door temperature potentially being beyond 8°C. The assumption is supported by the data compiled by European International Diabetes Federation (18). In general, it is recommended to keep in-use insulin at room temperature. However the "room temperature" mentioned usually means not exceeding 25°C although it is allowed to be up to 30°C (7). Clearly, in the context of tropical or hot climate countries, this standard is not met.

Pharmacists and other health professionals may have rarely been aware of actual household storage temperature of insulin. Likewise, patients themselves might not be knowledgeable about the appropriate storage temperature other than following the advice on where to keep insulins. It was uncommon for the patients to check their insulin storage temperature (14, 19). The results of the present study added to the existing evidence that there was a gap in patient education about insulin storage.

Under the isothermal simulated temperature of over 40 ºC, potency of NPH and its premixed 30/70 insulins was retained in the acceptable 95-105% range, at week 3 and 4, respectively. RI appeared thermo-stable till week 2 as 2 of 3 batches did not pass the stability test at week 3. Thus retention of potency might not be totally reassuring for RI kept under over 40°C beyond 2 weeks. Each insulin product under the stability test in the study was from the same manufacturer with the same year of production. They were no more than

1 year after manufacture. Each batch of each insulin product was produced no more than 3 months apart. Thus it can be confident that the results of the assay were not confounded by variations of the products tested. From the practice point of view, in the summer time where temperature could be high, if the use of RI was not finished within 14 days, it may be wise to keep the product in the fridge even 2-8 ºC was not achievable or not ascertained. Similar recommendation could be applied to in-use NPH beyond 21 days. Changes in other physical characteristics namely pH and presence of precipitates as well as clarity were negligible or absence, respectively, for all 3 insulin types. Acidic pH of < 5 and alkali pH of > 8 rapidly induced degradation of insulin, with different mechanisms (20). It could be inferred from the present study that pH of the human insulin products were not significantly affected by the exposure to over 40 ºC temperature.

Of note, NPH and its premixed suspension appeared viscous via visual observation which was further confirmed with difficulty in drawing the samples with the syringe needle. The finding was unanticipated. It has not been known if the increased viscosity affected the properties of insulin and other constituents in the products. In actual practice, this would take time to roll the cartridge to make the suspension distributed uniformly before injection. The viscosity might also affect a precise delivery of insulin when injected.

The results of insulin assay in the present study may not be directly compared to those in the other studies, due to differences in 1) the simulated temperature setting, i.e., fluctuating or isothermal conditions and degree of temperature under which insulin was placed; 2) types of insulin studied; 3) whether insulin samples were imitating daily usage (in-use) or representing unopened insulins. All insulins in the present study were representative of in-use human insulin products. The only published study in Thailand (15) reported that the contents of opened basal insulins in cartridge for use with pen were retained after 4 weeks under the cycling temperature of 37°C. The study in Kenya (21) demonstrated that insulins similar to those used in the present study, either unused or opened, had the contents acceptably maintained at 4 weeks under fluctuating temperatures in the range of 25 to 37°C. That study also indicated that the in vitro blood glucose lowering effect of the insulins was observed. They also showed that RI and NPH insulins were stable at continuous 37°C for 1 week. This was in contrast with the present study which reported more durable thermostability of similar insulins up to 2 and 3 weeks, respectively under isothermal higher temperature (42±2°C). It is not known if RI and NPH insulins in their study were representatives of spare or opened insulin. Up to present, thermostability studies on insulin potency applied as high temperature as 37°C only (22). The present study provided real life data on storage temperature of in-use insulins at patients' home. A new insight was added into insulin stability under more extreme temperature obtained from the patient domestic setting. Although the study was from a single site in one geographical area, it could be generalized to other similar tropical region.

The study has some limitations. Room temperature profile obtained could be affected by the season in which the study was carried out. The simulated temperature setting was isothermal which did not reflect the actual up and down pattern of temperatures in real life situations. The study did not ascertain the presence of insulin degradation, i.e., insulin fibrillation, under such a high temperature. The degraded product when injected can cause adverse effects, and biological activity of insulin may also be reduced (23-24). Evaluating glucose lowering activity of insulin products exposed to the high temperature as in the present study is required. Finally, the study did not ask the participants to provide the exact storage location in their home. The data could be useful for future patient education.

Based on the findings, health care professionals including pharmacists in hot climate countries should exercise discretion on the recommendation of in-use insulins storage at room temperature. To individualize the advice, healthcare professionals may have to consider seasonal maximum temperatures in their contexts as well as the number of days it takes for one insulin cartridge to be finished.

Conclusion

Patients with diabetes kept their in-use human insulin products in the fridge and at room temperature. The storage temperatures were mostly out of the recommended range, especially of the room temperatures which totally exceeded the acceptable 30°C. In-use RI, NPH as well as their premixed product (30/70), for use with a self-assembly pen were thermostable with the percentage label amount of insulin being in 95-105% range, under the temperature as high as 42 ±2°C for 2, 3 and 4 weeks, respectively.

Acknowledgements

The authors wish to express gratitude to Drs. Chawanee Thongpanchang and Jittra Kornsakulkarn, from the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand for their facility support, and Assoc Prof Dr Jinda Wangboonskul for sharing her valuable expertise in HPLC. Our sincere thank also extends to outpatient pharmacists at Thammasat University Hospital for helping in patient recruitment. All laboratory facilities were supported by the Faculty of Pharmacy, Thammasat University (Rangsit center). Special thanks go to Mr. Harvey Taylor for language proofreading.

References

1. American Diabetes Association Professional Practice Committee. 9. Pharmacological approaches to glycemic treatment: Standards of medical care in diabetes 2025. Diabetes Care 2025; 48(suppl 1):S181-S206.
2. National Drug Policy Division. National List of Essential Medicines [onlne]. 2024 [cited Mar 12, 2025]. Available from: ndp.fda.moph.go.th/nlem/67-1.
3. European Medicines Agency. ICH harmonised tripartite guideline for the stability testing of new drug substances and products, ICH topic q5c quality of biotechnological products: stability testing of biotechnological/ biological products [online]. 1996 [cited Sept 19, 2024]. Available from: www.ema.europa.eu/documents/scientific-guideline/ich-topic-q-5-c-quality-biotechnological-products-stability-testing-biotechnological/biological-products_en.pdf.
4. Moslemi P, Najafabadi AR, Tajerzadeh H. A rapid and sensitive method for simultaneous determination of insulin and A21- desamido insulin by high-performance liquid chromatography. J Pharmaceut Biomed Anal 2003; 33: 45-51.
5. Wang W, Singh SK, Li N, Toler MR, King KR, Nema S. Immunogenicity of protein aggregates--concerns and realities. Int J Pharm 2012; 431: 1-11. doi: 10.1016/j.ijpharm.2012.04.040.
6. Heinemann L, Braune K, Carter A, Zayani A, Kramer LA. Insulin storage: A critical reappraisal. J Diabetes Sci Technol 2021; 15: 147–59.
7. U.S. Food and Drug administration. Information regarding insulin storage and switching between products in an emergency [online]. 2017 [cited Feb 13, 2024]. Available from: www.fda.gov/drugs/emergency-preparedness-drugs/information-regarding-insulin-storage-and-switching-between-products-emergency.
8. Braune K, Kraemer LA, Weinstein J, Zayani A, Heinmann L. Storage conditions of insulin in domestic refrigerators and when carried by patients: often outside recommended temperature range. Diabetes Tech Therapeut 2019; 21: 238-44.

9. Thai Meteorological Department. Annual mean maximum temperature in Thailand [online]. 2022 [cited Mar 12, 2025]. Available from www.tmd.go.th/en/ClimateChart/annual-mean-maximum-temperature-in-thailand.
10. Climate Change Research Strategies Center. Climate change in Thailand. Bangkok; National Research Council of Thailand; 2022.
11. Ogle GD, Abdullah M, Mason D, Januszewski AS, Besançon S. Insulin storage in hot climates without refrigeration: Temperature reduction efficacy of clay pots and other techniques. Diabet Med. 2016; 33: 1544–53.
12. Taerahkun S, Sriphrapradang C. Efficacy of alternative cooling devices used for insulin storage without refrigeration under hot-humid environment. Ann Med 2022; 54: 1118-25.
13. Kimaro E, John J, Damiano P, Konje ET, Mori AT. Kidenya BR., et al. Impact of insulin storage and syringe reuse on insulin sterility in diabetes mellitus patients in Mwanza Tanzania. Sci Rep 2025; 15: 6232. doi.org/10.1038/s41598-025-91029-5.
14. Kongmalai T, Sriwijitkamol A. Evaluation of Thai Insulin-treated diabetics' knowledge and practice of insulin pen storage. J Med Assoc Thai 2019; 102: 503-7.
15. Kongmalai T, Preechasuk L, Junnu S, Manocheewa S, Srisawat C, Sriwijitkamol A. The effect of temperature on the stability of in- use insulin pens. Exp Clin Endocrinol Diabetes. 2021; 129: 683-8.
16. United States Pharmacopeia. Insulin human injection. In: United States Pharmacopeial Convention. USP42-NF 37. Rockville, MD; 2019, p. 2299.
17. Pendsey S, James S, Garrett TJ, Nord AB, Pendsey S, Malmodin D, et al. Insulin thermostability in a real-world setting. Lancet Diabetes Endocrinol 2023; 11: 310-2. doi.org/10.1016/S2213-8587(23)00028-1.
18. Krämer L, Vlasenko I. Storage of insulin [online]. 2019 [cited Feb 18, 2022]. Available from:. idf.org/images/IDF_Europe/Storage_of_Insulin_-_IDF_Europe_Awareness_Paper_-_FINAL.pdf.
19. Nitschke E, Heinemann L, Cartner A, Krämer LA, Braune K. What do healthcare professionals and people with diabetes know about insulin transport and storage? A multinational survey. J Diabetes Sci Technol 2021; 15: 719–22.
20. Brange J, Langkjaer L. Chemical stability of insulin. 3. Influence of excipients, formulation, and pH. Acta Pharm Nord. 1992; 4: 149-58.
21. Kaufmann B, Boulle P, Berthou F, et al. Heat-stability study of various insulin types in tropical temperature conditions: New insights towards improving diabetes care. PLoS ONE 2021; 16: e0245372.
22. Richter B, Bongaerts B, Metzendorf MI. Thermal stability and storage of human insulin. Cochrane Database of Systematic Reviews 2023, Issue 11. Art. No.: CD015385. doi:10.1002/ 4651858. CD015385.pub2
23. Brange J, Havelund S, Hougaard P. Chemical stability of insulin. 2. Formation of higher molecular weight transformation products during storage of pharmaceutical preparations. Pharm Res 1992; 9: 727–34.
24. Nakamura M, Misumi Y, Nomura T, Oka W, Isoguchi A, Kanenawa K et al. Extreme adhesion activity of amyloid fibrils Induces subcutaneous insulin resistance. Diabetes 2019; 68: 609-16.