ORIGINAL ARTICLE

Stress stability testing of Insulin Injection: A new perspective on enhancing diabetes management

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Abstract

Introduction: Recommended storage for insulin injection (unopened containers) is as "Store in a refrigerator (36°F–46°F [2°C–8°C]), but not in the freezer. Do not use if it has been frozen. If stored at room temperature, below 86°F (30°C) the vial must be discarded after 31 days. Strict storage recommendations for insulin are difficult to follow in hot tropical regions and even more challenging in conflict and humanitarian emergency settings, adding an extra burden to the management of people with diabetes. **Objectives:** According to pharmacopeia, unopened insulin vials must be stored in a refrigerator (2°C–8°C), while storage at ambient temperature (25°C–30°C) is usually permitted for the 4-week usage period during treatment. In the present work, we address a critical question toward improving diabetes care in resource poor settings, namely, whether insulin is stable and retains biological activity in tropical temperatures during a 4-week treatment period. To answer this question, we studied temperatures in tropical conditions in various regions such as Rajasthan, parts of Gujarat and Haryana in India. Oscillating temperatures between 25°C and 37°C were observed in various regions. **Experimental:** Insulin heat stability was assessed under these specific temperatures which were precisely reproduced in the laboratory using simulating storage chambers at 50°C, 40°C, 30°C, 25°C, and as a control 2°C–8°C. The stability of commercially available formulations of insulin was confirmed across the assessment period (4 weeks) as it exhibited flawless adherence to pharmacopeial guidelines during weekly high-performance liquid chromatography quantifications. **Results:** The evaluation of insulin efficiency showed that the samples held at different temperatures over the use period had the same insulin bioactivity as the samples kept at 2°C–8°C. When seen collectively, these findings show that insulin may be kept at such fluctuating ambient temperatures throughout the typical 4-week usage period when the patients are unable to find a storage temperature mentioned in label. **Conclusion:** It is now feasible to control diabetes more easily in settings with low resources and in humanitarian circumstances by removing the barrier of cold storage while in use.

Key words: Heat-stability of Insulin, peoples with diabetes, tropical

INTRODUCTION

Millions of vials, pens, and reservoirs of insulin are currently stored or in transit in refrigerated or unrefrigerated distribution centers, pharmacies, hospitals, delivery vehicles, homes, bathroom counters, pockets, purses, backpacks, belt clips, lockers, glove compartments, and planes. It is astonishing that very little published research seems to have been conducted that explores the potency of insulin doses at various steps of the cold chain up to the point that they are administered.

Insulin, like many other peptide hormone-based drugs, is temperature sensitive and its stability is affected by storage conditions. This is reflected in the resources invested in cold chain maintenance in the insulin supply chain and the specific storage recommendations that apply for insulin when in the hands of people with diabetes (PwD). Insulin, among other temperature-sensitive medications, presents a particularly unique case due to the exposure to environmental factors

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Table 1: Summarized data of analysis of insulin injection, biphasic isophane formulation, and heat-stability study at 2°C–8°C

Product name	Insulin injection, biphasic isophane 40 IU/mL, 10 mL vial (30:70)
Brand name	Biosulin 30:70
Batch number	TB71181222
Mfg. date	12.2022
Exp. date	11.2024
Temperature condition (sample stage)	

S. No.	Test	Specification	Initial	2°C–8°C	
				14D	28D
1	Impurities with molecular mass greater than that of Insulin (HMWP)	Not more than 3.0%	0.46	0.5	0.5
2	Assay (L.C.=40 IU/mL)	Between 90.0% and 110.0% of L.C.	101.9	100.1	101.3
3	Related proteins				
	A-21 desamido-insulin content	Not more than 5.0%	0.4	0.5	0.6
4	Sum of the area of any other peaks	Not more than 6.0%	0.5	0.4	0.3
5	Meta-cresol content (L.C.: 0.16% w/v)	Between 80.0% and 120.0% of L.C.	99.7	94.4	93.9
6	Phenol content (L.C.: 0.065%w/v)	Between 80.0% and 120.0% of L.C.	105	98.3	97.8
	Inferences →	Formulation complies to stability indicating tests up to 28 days at 2°C–8°C			

Table 2: Summarized data of analysis of insulin injection, biphasic isophane formulation, and heat-stability study at 25°C

Product name	Insulin injection, biphasic isophane 40 IU/mL, 10 mL vial (30:70)
Brand name	Biosulin 30:70
Batch number	TB71181222
Mfg. date	12.2022
Exp. date	11.2024
Temperature condition (sample stage)	25°C

Serial number	Test	Specification	Initial	25°C	
				14 D	28 D
1	Impurities with molecular mass greater than that of Insulin (HMWP)	Not more than 3.0%	0.46	0.5	0.5
2	Assay (L.C.=40 IU/mL)	Between 90.0% and 110.0% of L.C.	101.9	100.1	101.3
3	Related proteins				
	A-21 Desamido-insulin content	Not more than 5.0%	0.4	0.5	0.6
	Sum of the area of any other peaks	Not more than 6.0%	0.5	0.4	0.3
4	Meta-cresol content (L.C.: 0.16% w/v)	Between 80.0% and 120.0% of L.C.	99.7	94.4	93.9
5	Phenol content (L.C.: 0.065%w/v)	Between 80.0% and 120.0% of L.C.	105	98.3	97.8
	Inferences →	Formulation complies to stability indicating tests up to 28 days at 25°C			

when in-use. Precise dosing is a key in intensive insulin therapy. PwD s around the world carry insulin with them daily, in vials, pens, or pumps close to their body and store their insulin supply in household refrigerators. Insulin is regularly subjected to risk factors that can affect its potency when in-use, such as high and low ambient temperatures, sun light, and agitation through movement. In insulin pumps worn close to the body, not only is the temperature in the reservoir increased the constant movements also accelerate fibril formation. Many PwD live in places without access

Table 3: Summarized data of heat stability study at 30° cofinsulin injection, biphasic isophane for mulation, and heat-stability study at 30°C

Product name			Insulin injection, biphasic isophane 40 IU/mL, 10 mL vial (30:70)								
Brand name			Biosulin 30:70								
Batch number			TB71181222 [40IU]								
Mfg. date			12.2022								
Exp. date			11.2024								
Temperature condition (sample stage)			30°C								
Serial number	**Test**	**Specification**	**Initial**	**3 D**	**7 D**	**10 D**	**14 D**	**21 D**	**28 D**	**45 D**	**60 D**
1	Impurities with molecular mass greater than that of insulin (HMWP)	Not morethan3.0%	0.46	0.7	0.64	0.74	0.9	1.4	1.6	2.1	2.0
2	Assay (L.C.=40IU/mL)	Between 90.0% and110.0% of L.C.	101.9	101.1	101.1	101.7	98.9	100.4	100.8	100.9	99.5
3	Related proteins										
3a	A-21 Desamido-insulin content	Not more than 5.0%	0.4	0.5	0.5	0.6	0.5	0.6	0.7	0.4	0.5
3b	Sum of the area of any other peaks	Not more than 6.0%	0.5	0.7	0.9	1.1	0.7	0.6	0.8	1.3	1.7
4	Meta-cresol content (L.C.: 0.16% w/v)	Between 80.0% and 120.0% of L.C.	99.7	94.0	92.5	91.7	94.4	93.7	94.4	94.4	97.1
5	Phenol content (L.C.:0.065% w/v)	Between 80.0% and 120.0% of L.C.	105.0	96.3	93.3	93.4	98.6	97.1	97.9	98.7	97.3
Inferences →		Formulation complies to stability indicating tests up to 60 days at 30°C.									

to refrigeration, and recent data indicate that even insulin storage conditions in household refrigerators often do not meet recommendations by manufacturers. Exact dosing is essential for PwD to maintain glycemic control.[1,2] PwDs perceive changes in insulin sensitivity often and need to adjust their dose accordingly. Changes in insulin potency can contribute to this observed variability in glucose levels; however, this factor is currently not sufficiently considered. Laboratory analysis is needed to verify if insulin potency has been affected, making it difficult to assess in practice. Furthermore, there are little publicly available data on insulin stability.[3-6] It is complicated for PwD to evaluate which risks that they face when using insulin that has or might have been stored outside of recommendations. This review summarizes what is known about the storage of insulin, how it affects insulin stability, current practice in the distribution chain and when stored by PwD and discusses how the factor of insulin potency could be taken into account to improve diabetes management.

MATERIALS AND METHODS

Materials

Studied insulin formulation

Examined the formulations of insulin in addition to the human insulin formulations utilized, commercial insulin formulations of MJ BioPharm, that is, insulin injection, biphasic isophane 40 IU/mL, and 10 mL vial (30:70). The brand name is Biosulin 30:70 (Batch No. TB71181222).

STUDY DESIGN: (METHODOLOGY)

To assess insulin stability, the temperature oscillations that were observed in the field were replicated in the laboratory and insulin formulation was exposed to these fluctuating temperatures in the laboratory. A mixed insulin vial was

Table 4: Summarized data of heat-stability study at 40° C of Insulin in injection, biphasic isophane formulation and Heat stability study at 40°C

Product name			Insulin injection, biphasic isophane 40 IU/mL, 10 mL vial (30:70)							
Brand name			Biosulin 30:70							
Batch number			TB71181222 [40IU]							
Mfg. date			12.2022							
Exp. date			11.2024							
Temperature condition (sample stage)			40°C							
Serial number	**Test**	**Specification**	**Initial**	**3D**	**7D**	**10D**	**14D**	**21D**	**28D**	**45D**
1	Impurities with molecular mass greater than that of insulin (HMWP)	Not more than 3.0%	0.46	0.7	1.08	1.28	1.7	2.2	2.6	3.57
2	Assay (L.C.=40 IU/mL)	Between 90.0% and110.0% of L.C.	101.9	100.9	99.7	98.8	97.6	98.8	98.3	97.5
3	Related proteins									
3a	A-21 Desamido-insulin content	Not more than 5.0%	0.4	0.5	0.5	0.1	0.5	0.6	0.6	0.4
3b	Sum of the area of any other peaks	Not more than 6.0%	0.5	0.6	1.1	1.1	1.0	1.2	1.3	2.0
4	Meta-cresol content (L.C.:0.16% w/v)	Between 80.0% and 120.0% of L.C.	99.7	94	91.6	91.3	93.8	93.2	94.3	94.3
5	Phenol content (L.C.:0.065% w/v)	Between 80.0% and 120.0% of L.C.	105.0	96.5	93.3	93.2	98	96.9	97.8	98.8
Inferences →		Formulation complies all tests to elevated temperature conditions up to 28 days at Mention RH as below along with temperature throughout documents: 25°C/60%RH 30°C/65%RH 40°C/75%RH.								

stored at ambient temperature in simulating chamber during a period of 4 weeks and withdrawn for analysis. Insulin stored at 2°C–8°C was used as positive control and heat-degraded insulin as negative control. Furthermore, the effect of continuous exposure to high temperatures of 25°C, 30°C, 40°C, and 50°C were also investigated.[7,8]

Four parameters were investigated in the insulin formulations (Biphasic isophane insulin formulation): (i) potency determination by liquid chromatographic method (high-performance liquid chromatography [HPLC]), which is the gold standard method for insulin stability assessment according to major pharmacopeia's, (ii) impurities with molecular mass greater than that of insulin (high-molecular-weight product [HMWP]) determination by High performance liquid chromatographic method (HPLC), which is the key degradant in insulin formulation, (iii) related proteins determination by High performance liquid chromatographic method (HPLC) which is the second key degradant in insulin formulation, and (iv) preservative contents determination a. meta-cresol and b. phenol determination by High performance liquid chromatographic method (HPLC) which are essential to preserve the insulin formulation from microbial rise while using period.[9-11]

METHODS

Insulin Quantification and Potency Determination by High performance liquid chromatographic method (HPLC)

According to pharmacopeia's, reversed-phase HPLC coupled with ultraviolet detection at 214 or 280 nm is the method of choice for insulin quantification and for potency determination due to the correlation that has been described between HPLC quantification and biological activity. For the testing method of potency determination of insulin, refer monograph of Indian pharmacopoeia-2022.

Impurities with Molecular Mass Greater Than that of Insulin (HMWP) Determination by High performance liquid chromatographic method (HPLC)

For the testing method of impurities with molecular masses greater than that of insulin, refer monograph of Indian pharmacopoeia-2022.

Table 5: Summarized data of heat stability study at 50°C of insulin injection, biphasic isophane formulation, and heat-stability study at 50°C

Product name			Insulin injection, biphasic isophane 40 IU/mL, 10 mL vial (30:70)						
Brand name			Biosulin 30:70						
Batch number			TB71181222 [40IU]						
Mfg. date			12.2022						
Exp. date			11.2024						
Temperature Condition			50°C						
Serial number	**Test**	**Specification**	**Initial**	**3D**	**7D**	**10D**	**14D**	**21D**	**28D**
1	Impurities with molecular mass greater than that of insulin (HMWP)	Notmorethan3.0%	0.46	0.91	0.85	1.48	1.7	2.6	3.2
2	Assay (L.C.=40 IU/mL)	Between 90.0% and 110.0% of L.C.	101.9	101.1	99.5	97.3	96.6	99.1	94.4
3	Related proteins								
3a	A-21 Desamido-insulin content	Not more than 5.0%	0.4	0.5	0.5	0.5	0.4	0.5	0.6
3b	Sum of the area of any other peaks	Not more than 6.0%	0.5	0.7	1	1.7	1.1	1.7	2
4	Meta-cresol content (L.C.: 0.16% w/v)	Between 80.0% and 120.0% of L.C.	99.7	95.9	91.9	92.3	93.7	93.1	94.3
5	Phenol content (L.C.:0.065% w/v)	Between 80.0% and 120.0% of L.C.	105.0	98.1	93.7	94.1	98.0	96.8	98.3
Inferences →		Formulation complies to stability indicating tests up to 21 days at 50°C in all test parameters							

Related Proteins Determination by High performance liquid chromatographic method (HPLC)

For the testing method of related proteins determination, refer monograph of Indian pharmacopoeia-2022.

Preservative Contents Determination

a. Meta-cresol
b. Phenol

Preservative contents determination meta-cresol and phenol determination by High performance liquid chromatographic method (HPLC).

Determination of Meta-Cresol by High performance liquid chromatographic method (HPLC)

The Instruments required for practical procedure are HPLC, analytical balance, and pH meter. The chemical/reagent required is anhydrous sodium sulfate, phosphoric acid, ethanolamine, acetonitrile, and hydrochloric acid. The M-cresol WS and phenol WS are required as reference.[12-14]

Standard/working standard required for the practical procedure

Chromatographic conditions are maintained for HPLC as column is ODS, 5 µm with 4.6 mm × 250 mm of diameter. The selected wavelength was 214 nm and flow rate of solvent was 1 mL/min with injection volume of 20 µl and column temp was maintained 40°C. The peak was observed by considering maximum runtime within 35 min.

Mobile phase A: (Buffer pH 2.3)

Weigh 28.4 g of anhydrous sodium sulfate and dissolved it in 1000 mL of water, then add 2.7 mL phosphoric acid, and adjust pH of 2.3 ± 0.05 with ethanolamine, filtered and degassed.

Mobile phase B

Buffer pH 2.3 and acetonitrile are mixed in proportion 550:450. In case of necessary, correction is carried out.

Mobile phase mixture

42 and 58 volumes of mobile phase A and B, respectively.

Standard

Weight about 160 mg of meta-cresol WS in a 100 mL volumetric flask add about 50 mL 0.01M HCI and sonicate for 2 min dilute up to 100 mL mark with 0.01M HCl further dilute 5–50 mL 0.01M hydrochloric acid.

Test solution: (To be prepared sample in duplicate)

Shake well the composite sample to make a homogeneous and pipette out 5 mL of the test sample from the composite sample and transfer into a 50 mL volumetric flask and dilute it up to the mark with 0.01M HCI.

Acceptance criteria

Between 0.128% w/v and 0.192% w/v and between 80.0% and 120.0% of L.C.

Determination of Phenol by High performance liquid chromatographic method (HPLC)

The instruments required for practical procedure are HPLC, analytical balance, and pH meter. The chemical/reagent required is anhydrous sodium sulfate, phosphoric acid, ethanolamine, acetonitrile, and hydrochloric acid. The M-cresol WS and phenol WS are required as reference.

Standard/working standard required for the practical procedure

Chromatographic conditions are maintained for HPLC as column is ODS, 5 µm with 4.6 mm × 250 mm of diameter. The selected wavelength was 214 nm and flow rate of solvent was 1 mL/min with injection volume of 20 mL and column temp was maintained 40°C. The peak was observed by considering maximum runtime within 35 min.

Mobile phase A: (Buffer pH 2.3)

Weigh 28.4 g of anhydrous sodium sulfate and dissolved it in 1000 mL of water, then add 2.7 mL phosphoric acid, and adjust pH of 2.3 ± 0.05 with ethanolamine, filtered and degassed.

Mobile phase B

Buffer pH 2.3 and acetonitrile are mixed in proportion 550:450. In case of necessary, correction is carried out.

Mobile phase mixture

42 and 58 volumes of mobile phase A and B, respectively.

Standard

Weight about 65 mg of phenol WS in a 100 mL volumetric flask add about 50 mL 0.01M HCI and sonicate for 2 min dilute up to 100 mL mark with 0.01M HCl further dilute 5–50 mL 0.01M hydrochloric acid.

Test solution: (To be prepared sample in duplicate)

Shake well the composite sample to make a homogeneous and pipette out 5 mL of the test sample from the composite sample and transfer into a 50 mL volumetric flask and dilute it up to the mark with 0.01 M HCI.

Acceptance criteria

Between 0.052% w/v and 0.078% w/v and between 80.0% and 120.0% of L.C.

RESULTS

First, analysis was done for initial batch samples of insulin formulations and results are compiled in Table 1 as a standard value to compare further potency loss and degradation path for all other elevated temperature storage conditions.[15-17]

Evaluation of Insulin Injection, Biphasic Isophane at Real Time Storage Conditions, that is, 2°C–8°C

First, analysis has been carried out for stability storage condition of insulin formulations, that is, 2°C–8°C as a control sample result to compare with thermally excursed insulin formulations. Results were presented for 14 and 28 days at 2°C–8°C, as shown in Table 1.

Evaluation of Insulin Injection, Biphasic Isophane at accelerated storage condition, that is, 25°C

The stability of insulin formulation was evaluated on 14th and 28th day at 25°C as it is an accelerated stability storage condition. Results were presented for 14 and 28 days at 25°C, as shown in Table 2.

Evaluation of Insulin Injection, Biphasic Isophane at Accelerated Storage Condition, that is, 30°C

Further insulin formulation was studied at elevated temperature storage conditions. The stability sample was checked on 3rd, 7th, 10th, 14th, 21st, and 28th day at 30°C. Data showed no significant change. Results are presented in below Table 3.

Evaluation of Insulin Injection, Biphasic Isophane at elevated temperature storage condition, that is, 40°C

Further insulin formulation was studied at exaggerated temperature storage condition. The stability sample was evaluated on 3rd, 7th, 10th, 14th, 21st, and 28th Day at 40°C.

Data showed no significant change in the results, as shown in Table 4.

Evaluation of Insulin Injection, Biphasic Isophane at Exaggerated Temperature Storage Condition, that is, 50°C

Insulin formulation was also tested at high temperature to check its potency on stability. Many regions of Asia now reached to the temperature of 40°C–45°C, Sub-Sahara region and Caribbean region already reached to temperature of more than 45°C. The storage condition off or mulation was tested at 50° Con Day3rd, 7th, 10th, 14th, 21st, and 28th Day and no deviation of the results as that of day results, as shown in Table 5.

RESULTS/CHROMATOGRAPHY PRESENTATION AT DIFFERENT TEMPERATURE CONDITIONS

First, analysis was done for initial batch samples of insulin formulations and results are compiled in Table 2 as a standard value to compare further potency loss and degradation path for all other elevated temperature storage conditions.

Evaluation of Insulin Injection, Biphasic Isophane at Real-Time Storage Conditions, that is, 2–8°C

First, analysis has done for stability storage condition of insulin formulations, that is, 2–8°C as a control sample results to compare with thermally excursed insulin formulations. Results were presented for 14 and 28 days at 2–8°C, as shown in Table 2 and Figure 1.

Evaluation of insulin injection, biphasic isophane at accelerated storage condition, that is, 25°C/60% RH:

The stability of insulin formulation was evaluated on 14th and 28th Day at 25°C with 60% of relative humidity as it is an accelerated stability storage condition.

Figures 2 and 3 showed no significant change in the graph. Results were presented for 14 and 28 days at 25°C/60% RH, as shown in Table 2 and Figure 2.

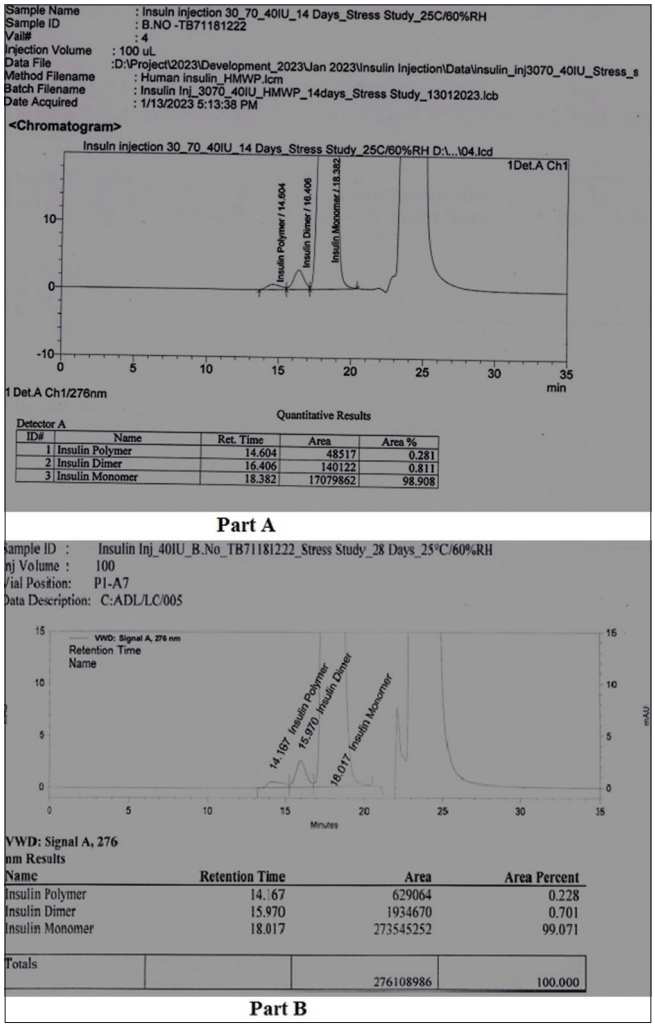


Figure 1: High-performance liquid chromatography graph of insulin injection, biphasic isophane at 2–8°C storage condition for 14 days and 28 days

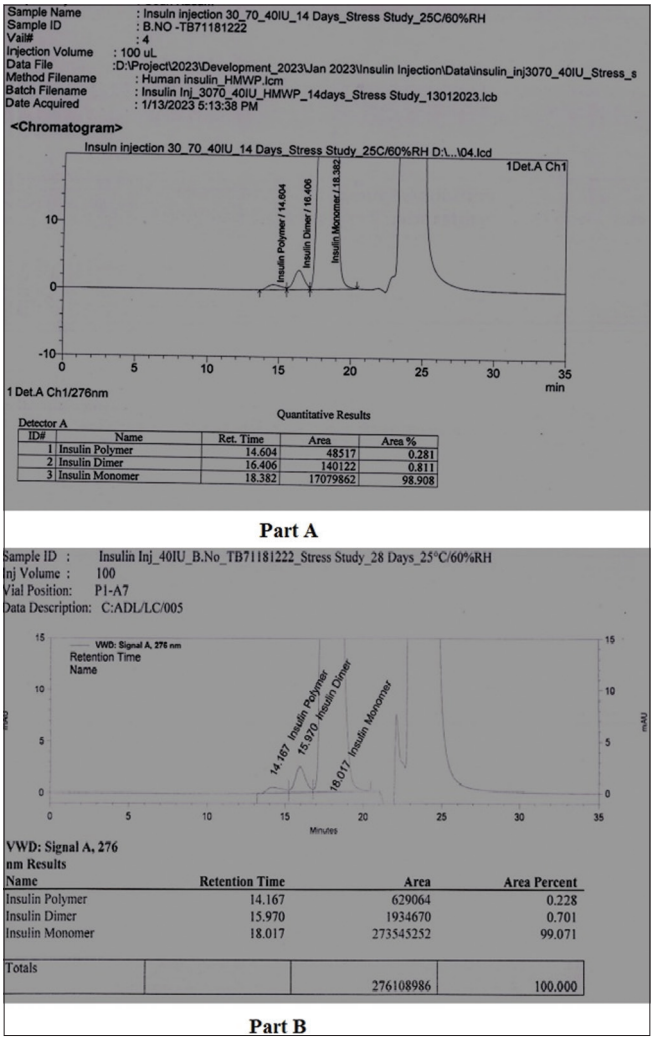


Figure 2: High-performance liquid chromatography graph of insulin injection, biphasic isophane at 25°C/60% RH storage condition to 14 and 28 days

Evaluation of Insulin Injection, Biphasic Isophane at accelerated storage condition, that is, 30°C/65% RH

Further insulin formulation was studied at elevated temperature storage condition. The stability sample was checked on 3rd, 7th, 10th, 14th, 21st, and 28th day at 30°C with 65% of relative humidity. It showed no significant change in the graph. Results are presented in the below Table 3 and Figure 3.

Evaluation of Insulin Injection, Biphasic Isophane at accelerated storage condition, that is, 40°C/75% RH

Further, insulin formulation was studied at elevated temperature storage condition. The stability sample was checked on 3rd, 7th, 10th, 14th, 21st, and 28th Day at 40°C with 75 % of relative humidity.

Figure 4 showed no significant change in the graph. Results are presented in the below Table 4 and Figure 4.

Evaluation of Insulin Injection, Biphasic Isophane at Extreme Elevated Temperature Storage Condition, that is, 50°C

Insulin formulation was also tested at high temperature to check its potency on stability. Many regions of Asia now reached to the temperature of 40–45°C, Sub-Sahara region and Caribbean region already reached to temperature of more than 45°C. The storage condition of formulation was tested at 50°C on Day 3rd, 7th, 10th, 14th, 21st, and 28th Day [Figure 5 and Table 5].

The initial analysis was carried out by same way and method according to storage condition and relative humidity as previously used for assay purpose. Due to limitation for publication of manuscript as a sample here mentioned only one graph which shows assay of insulin injection, biphasic isophane (30:70) 40 IU/mL in Figures 6 and 7 indicated 100% of assay result.

The test for detection of related protein was carried out on the insulin formulation as per the previous condition maintained

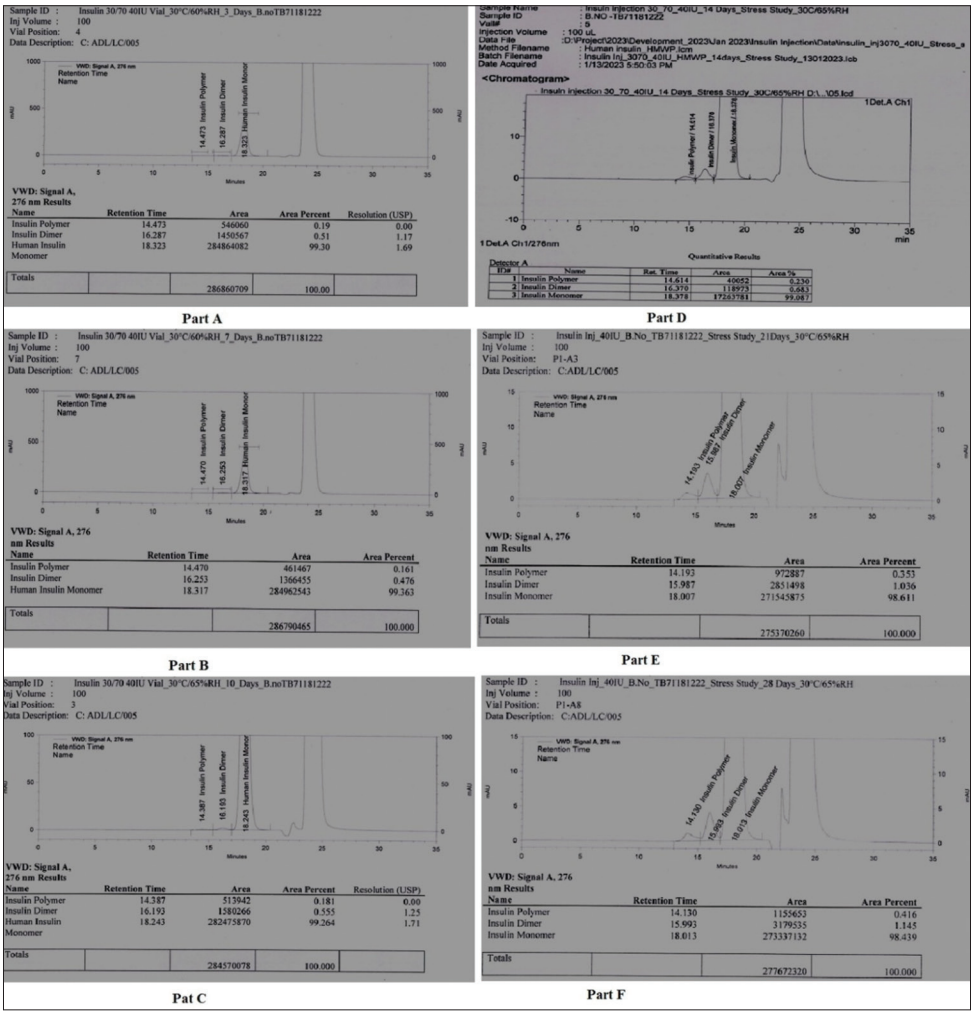


Figure 3: High-performance liquid chromatography graph of insulin injection, biphasic isophane at 30°C/65% RH storage condition for3 days (Part A), 7 day (Part B), 10 day (Part C), 14 day (Part D), 21 day (Part E), and 28 day (Part F)

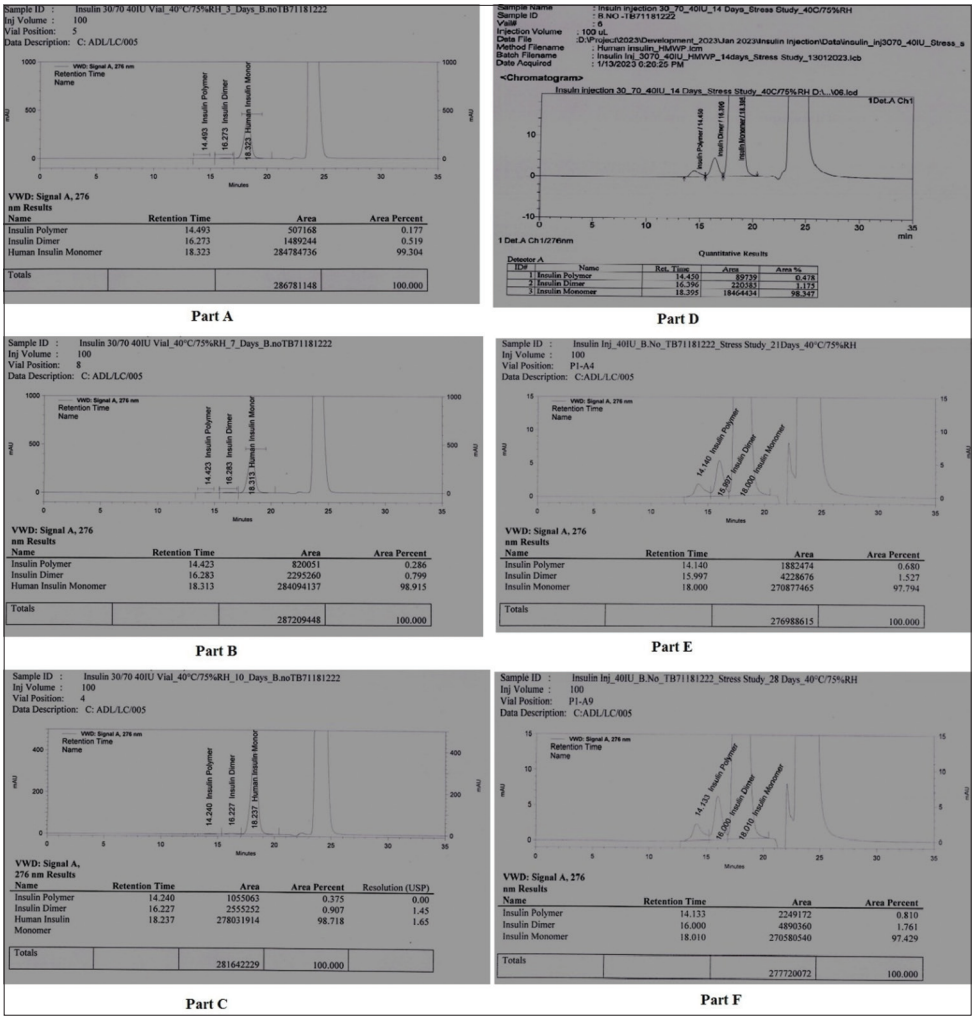


Figure 4: High-performance liquid chromatography graph of insulin injection, biphasic isophane at 40°C /75% RH storage condition for3 days (Part A), 7 day (Part B), 10 day (Part C), 14 day (Part D), 21 day (Part E), and 28 day (Part F)

for its storage and relative humidity with consecutive days. From the result, it was found that human insulin with retention time 22.943 and A-21 desomido human insulin 28.810 was found to be present as related proteins which were well within specification limits. Figure 9 indicates that the result of related protein was found within limit.

The initial analysis was carried out for the percent of preservative in the insulin biphasic isophane injection (30:70) 40 IU/mL. As per the procedure and previously mentioned, the injection contains phenol and meta-cresol as a preservative. The analysis was carried out with same storage condition and relative humidity. As a proof here mentioning only one graph for initial analysis in Figure 8 as a proof which indicates the presence of phenol at RT 10.413 and meta-cresol at RT 17.590.

DISCUSSION

The study presented here aimed at assessing the stability of insulin during its period of use by patients in regions presenting oscillating ambient temperatures which vary from those recommended by manufacturers. This study is focused on the usual 28 days of patient use of insulin, and it is clear that the storage conditions of insulin at the healthcare professional level should be maintained according to current recommendations, that is, adequate cold chain management from manufacturing until the point of delivery to patients.[18,19]

In this study, we analyzed the quality of insulin formulations stored under different conditions with respect to efficacy.

In this study, we have shown that commercial insulin formulations can be used in tropical temperature conditions similar to those studied for 4 weeks. All tested formulations remained in the acceptable range of insulin potency defined by pharmacopeia's, up to 4 weeks of thermal cycling from 25°C to 40°C, highlighting that the manufacturers' recommendations for insulin storage during the period of use by the patient are quite conservative.[20,21]

Thermal denaturation is a matter of both time and temperature and is a complex, partially reversible process. Increasing the

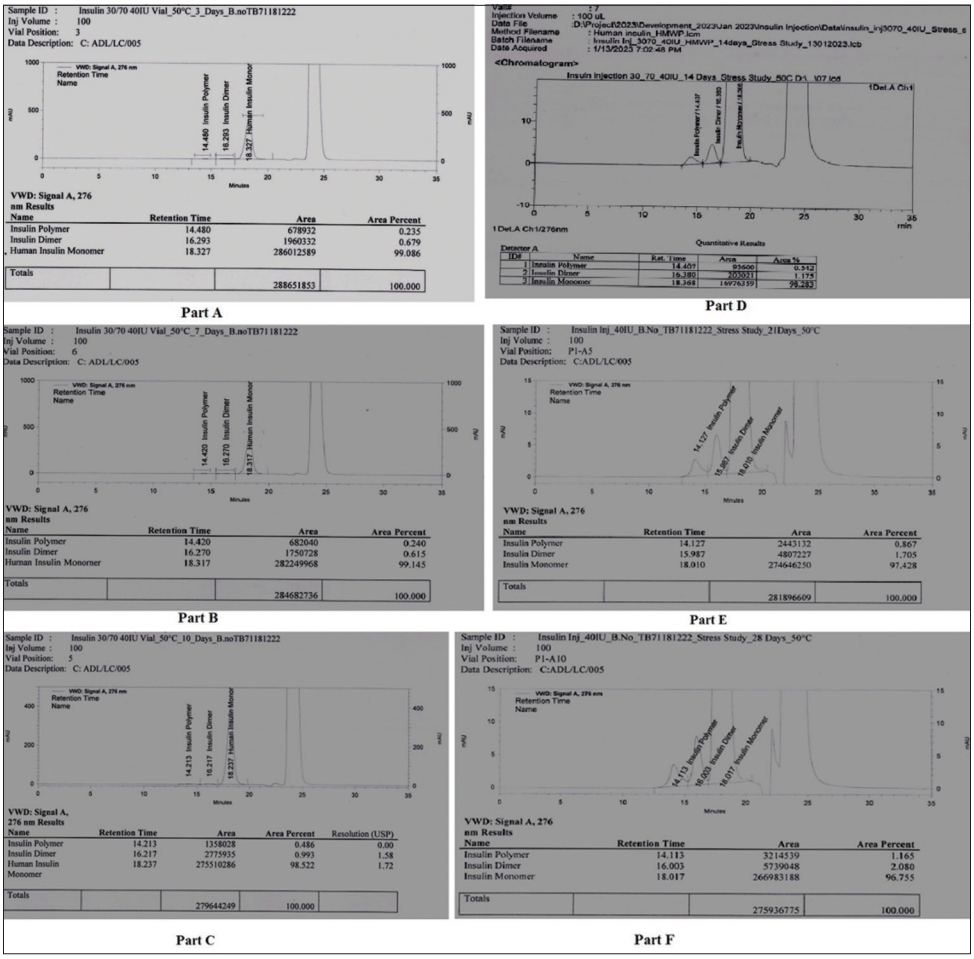


Figure 5: High-performance liquid chromatography graph of insulin injection, biphasic isophane at 50°C storage condition for 3 days (Part A), 7 day (Part B), 10 day (Part C), 14 day (Part D), 21 day (Part E), and 28 day (Part F)

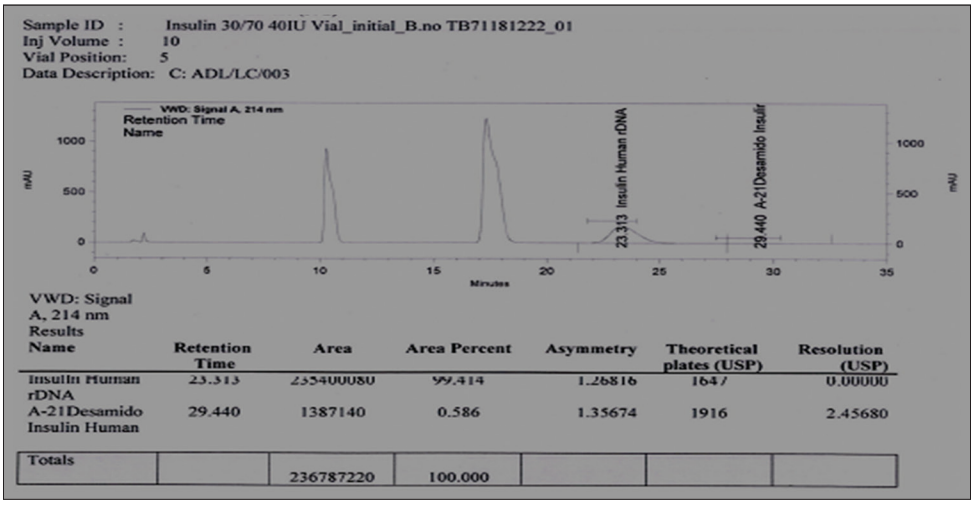
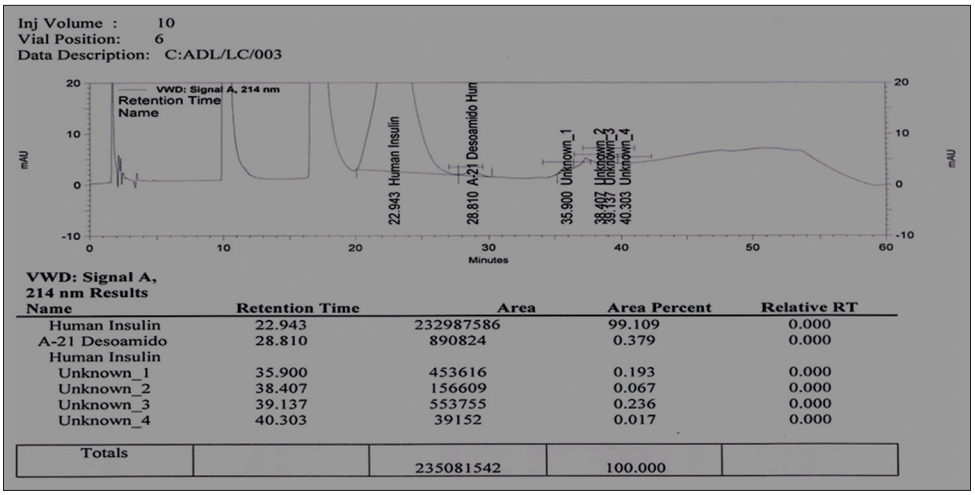


Figure 6: High-performance liquid chromatography graph of insulin injection, biphasic isophane for assay testing

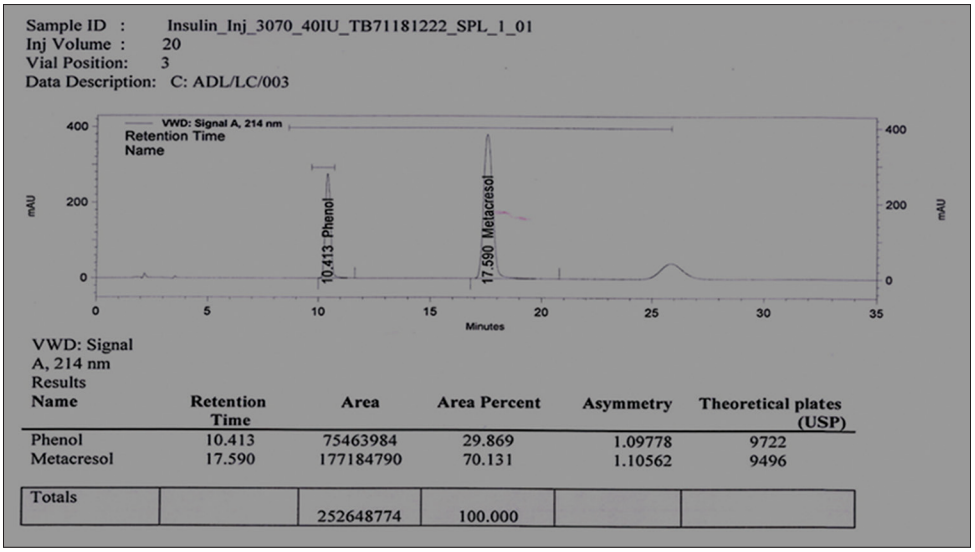
temperature can perturb the native protein conformation, which promotes unfolding of parts of the protein over time. It has been shown that partly unfolded proteins are more prone to aggregation than the native state of the protein. Thermal energy plays a role in surmounting the transition state. This thermally induced denaturation is time dependent and may be reversible for some proteins, but if sufficient energy is added to the system (such as application of high temperatures for a long time period), this usually leads to irreversible denaturation due to aggregation. The results obtained when insulin was continuously exposed to higher temperatures (40°C) over 4 weeks are in agreement with previously



VWD: Signal A, 214 nm Results

Name	Retention Time	Area	Area Percent	Relative RT
Human Insulin	22.943	232987586	99.109	0.000
A-21 Desoamido Human Insulin	28.810	890824	0.379	0.000
Unknown_1	35.900	453616	0.193	0.000
Unknown_2	38.407	156609	0.067	0.000
Unknown_3	39.137	553755	0.236	0.000
Unknown_4	40.303	39152	0.017	0.000
Totals		235081542	100.000	

Figure 7: Result of related protein was found within limit in insulin injection, biphasic isophane



VWD: Signal A, 214 nm Results

Name	Retention Time	Area	Area Percent	Asymmetry	Theoretical plates (USP)
Phenol	10.413	75463984	29.869	1.09778	9722
Metacresol	17.590	177184790	70.131	1.10562	9496
Totals		252648774	100.000		

Figure 8: High-performance liquid chromatography analysis of insulin injection, biphasic isophane for detection of phenol, and meta-cresol in the graph

released data on insulin heat stability. In contrast, significant degradation was not observed when the formulations were submitted to temperature cycling. During the temperature cycling process, the energy over time that is brought to the system is most likely not sufficient to lead directly to irreversible aggregation. Thus, a partially reversible unfolding of the protein can be hypothesized, avoiding nucleation and fibril formation. Whereas under continuous heating conditions, the amount of energy may be sufficient to promote irreversible conformational changes of the insulin molecules, leading to a nucleation process followed by fibril elongation. This is important as past publications and current tests on biological products are performed at isothermal temperatures and not using fluctuating temperatures. As such, current protocols should be extended to consider the reality of temperature fluctuations present in most settings where patients live, because this may have a big impact on recommendations for storage of biological products during the period of use.

CONCLUSION

Biosimilar insulins have the potential to reduce medication costs, increase accessibility to insulin therapy for patients, and increase the range of options from which insulin treatments can be chosen by physicians in collaboration with patients. However, the complexities of the manufacturing process may lead to final product variability, despite the similarity of amino acid sequence between a biosimilar and its reference product. Differences between originator and biosimilar insulins can potentially lead to efficacy and safety/immunogenicity consequences.

The results revealed no differences between originator and insulin drug products and possess excellent stability at its recommended storage condition 2°C–8°C. Furthermore, it is stable at 25°C since it is accelerated storage condition of drug product. The experimental temperature stressed studies demonstrate that insulin drug product is stable as per chemical stability at 30°C for

45 days. The experimental accelerated temperature stressed (40°C for 28 days) studies demonstrated that insulin drug products stability as this data are mandatory for the product approval.

The experimental temperature stressed studies demonstrate that insulin drug product is stable as per chemical stability at 50°C for 21 days. The investigation of further temperature ranges and the application of the findings to additional sites are ongoing tasks. The potential degradants like high-molecular-weight proteins found withing specified limits at the temperature conditions 40°C up to 28 days which is the recommended in-use storage period for the insulin formulations, it proves that the biosimilar formulation manufactured by MJ BioPharm is comparable in its antidiabetic medication qualities but more economic with respect to cost of the product and affordable to the most of the Indian population which are not able to keep biosimilar formulations at recommended storage conditions.

Antimicrobial protection is maintained throughout the study. Quantification of the preservatives present in the formulations was performed by HPLC and results at all the storage conditions and time period found within the specified limits. Hence, the formulation is microbe free while its in-use storage period.

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