

**Pharmaceutical Services Center
University of Tanta
College of Pharmacy
Tanta, Egypt.**

***Bioequivalence Study
of
Insulin H Mix 100 IU
Suspension
(30 / 70 mixture Human
Insulin & Protamine-
Insulin Human)
Produced by
SEDICO Pharmaceutical
Co., 6 October City -
Egypt.***



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Certificate

This is to certify that the product "**Insulin H Mix 100 IU**" suspension (30/70 mixture Human Insulin & Protamine Insulin Human), batch no. 0503101, manufacturing date 05/2003 and expiration date 11/2005, produced by SEDICO Pharmaceutical Co.,6 October City-Egypt, and the product "**Mixtard 30 HM**" suspension (Biphasic Isophane Insulin injection 30/70), batch no. NS60194, manufacturing date 11/2002 and expiration date 04/2005 manufactured by Novo Nordisk A/S, 2880 Bagsvaerd, Denmark are bioequivalent. This is because both drug products produce comparable therapeutic effect.

This is based on a study carried out on 24 healthy human volunteers for each product by the Pharmaceutical Services Center, College of Pharmacy, University of Tanta, Tanta, Egypt.

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Report synopsis:

Name of the Sponsor:

SEDICO Pharmaceutical Co., 6 October City, Egypt.

Name of the Finished Products:

a- *Test:* Insulin H Mix 100 IU suspension, batch no.0503101, manufacturing date 05/2003 and expiration date 11/2005, manufactured by SEDICO Pharmaceutical Co., 6 October City, Egypt.

b-*Reference:* Mixtard 30 HM suspension, batch no. NS60194, manufacturing date 11/2002 and expiration date 04/2005, manufactured by Novo Nordisk A/S, 2880 Bagsvaerd, Denmark.

Name of the Active Ingredient:

Human Insulin.

Title of the Study:

A two way, open – lable, randomized, single dose, cross-over study in twenty four healthy male volunteers to compare the therapeutic effect of the two different Insulin preparations.

Objectives of the study:

The objective of the study was to compare the therapeutic effect of Insulin commercial brands namely, Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human) manufactured by SEDICO Pharmaceutical Co., 6 October City, Egypt, and Mixtard 30 HM suspension (Biphasic Isophane Insulin injection 30/70) manufactured by Novo Nordisk A/S, 2880 Bagsvaerd, Denmark.

Study Center:

Pharmaceutical Service Center, College of Pharmacy, University of Tanta, Egypt.

This center has established a quality management system that is in conformance with the International Quality System Standards (ISO 9001) from ASR in the scope of bioequivalence studies.

Principal Investigator:

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Clinical Investigator:

Dr. Wael Farrag, MD, Departement of Internal Medicine, University of Tanta Hospital, Tanta, Egypt.

Number of Subjects:

Planned: 24

Analysed: 24

Diagnosis and Main Criteria for Inclusion:

Healthy, non-smoking, non-diabetic, male subjects, age 16 to 45 years with a normal body weight and body mass index.

Dosage & Administration:

A single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human) (SEDICO) or Mixtard 30 HM suspension (Biphasic Isophane Insulin injection 30/70) (Novo Nordisk).

Study Periods:

Date of the First period: 11/10/2003.

five days washout period

Date of the Second period: 16/10/2003.

Methodology:

Eligible subjects received test and reference preparation as a single subcutaneous injection on two different occasions, five days apart. Blood samples were collected before and at **15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes** after administration. Analysis of blood glucose concentrations in each sample by means of a photometric assay method was performed. Statistical comparisons of both preparations were based on the target therapeutic effect.

Analytical Methods:

A photometric assay method for the determination of glucose concentration in whole blood.

Criteria for Evaluation:

Pharmacokinetics: Not applicable.

*Pharmacodynamics :*Maximum reduction of blood glucose concentration, reduction in blood glucose concentration at 120 minutes, time of 50% maximum reduction in blood glucose concentration, area above the % of baseline blood glucose concentration-time curve.

(Test / Reference ratios had to be within the equivalence limit)

Statistical Methods:

The assessment of bioequivalence between the test and the reference products was based on the ratios of the log transformed mean of the pharmacodynamic parameters (maximum reduction of blood glucose concentration, reduction in blood glucose concentration at 120 minutes, time of 50% maximum reduction in blood glucose concentration, area above the % of baseline blood glucose concentration-time curve). Bioequivalence was concluded if either tail probability did not exceed the 90% confidence limit and was completely contained in the 0.80 – 1.25 range.

Limit of acceptance: For all the pharmacodynamic parameters:
80 to 125%.

Safety:

Pre-study

Physical examination, vital signs and laboratory screening tests.

Throughout the Study

Vital signs, adverse events

Conclusion:

The test drug product **Insulin H Mix 100 IU suspension** (30/70 mixture Human Insulin & Protamine Insulin Human) manufactured by SEDICO Pharmaceutical Co., 6 October City, Egypt, is bioequivalent to the reference brand **Mixtard 30 HM suspension** (Biphasic Isophane Insulin injection 30/70) manufactured by Novo Nordisk A/S, 2880 Bagsvaerd, Denmark.

Date of Final Report:

November, 17, 2003

Summary of Pharmacodynamic Parameters

Parameters	Insulin H Mix 100 IU (SEDICO)	Mixtard 30 HM (Novo Nordisk)
Maximum reduction in % of baseline blood glucose concentration (%)	34.06 ± 9.514	36.86 ± 8.653
Time of 50% maximum reduction in blood glucose concentration (mins)	57.50 ± 26.78	62.50 ± 25.66
Reduction in blood glucose concentration at 120 minutes (%)	26.65 ± 11.42	27.56 ± 10.31
Area above the % baseline blood glucose concentration-time curve (%.min)	5344 ± 1882	7314 ± 1419

Pharmacodynamic parameter comparison:

The 90% confidence limit of the difference between the log transformed mean values of:

- Maximum reduction in the blood glucose concentration for Insulin H Mix 100 IU and Mixtard 30 HM fall between **(93.66-99.32%)**
- Time corresponding to 50% of the maximum reduction in the blood glucose for Insulin H Mix 100 IU and Mixtard 30 HM fall between **(91.20-104.8%)**
- Reduction of blood glucose concentration at 120 minutes for Insulin H Mix 100 IU and Mixtard 30 HM fall between **(92.74-105.2%)**
- Area above the % of baseline blood glucose concentration – time curve for Insulin H Mix 100 IU and Mixtard 30 HM fall between **(82.67-89.27%)**

Comparative Bioequivalence Study of Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine - Insulin Human, SEDICO).

<u>List of Contents</u>	Page
1- Introduction	11
2- Objectives	13
3- Study Design	13
4- Study Medication	14
5- Study Protocol	
• Site and duration of the study	15
• Selection of volunteers	15
• Administration of the study drugs	15
• Dietary restriction	16
• Collection of blood samples	16
• Analysis of blood glucose concentration	17
• Pharmacodynamic calculations	18
• Statistical analysis	19
▪ Assessment of Bioequivalence	
6- Results:	
• In vivo biological evaluation.	
➤ Study population	20
➤ Withdrawals	20
➤ Results of health state of the volunteers	20
➤ Monitoring of the adverse drug reaction	20
➤ Biochemical results of the studied subject	21
➤ % of baseline blood glucose concentration-time data	21
• Clinical observation (Demographic and other baseline characteristic for volunteers)	22
• Biochemical results	23

• Individual data of % of baseline blood glucose concentration following a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 Human Insulin & Protamine – Insulin Human, SEDICO) to 24 healthy male volunteers.	25
• Individual data of % of baseline blood glucose concentration following a single subcutaneous injection (20 IU) of Mixtard 30 HM suspension (Biphasic Isophane Insulin 30/70 Novo Nordisk) to 24 healthy male volunteers.	26
• Pharmacodynamic results	52
• Statistical analysis of pharmacodynamic results	57
7- Discussion & Summary	62
8- Clinical Protocol & Report	
• Introduction	64
• Study Objectives	65
• Investigations	66
• Method and Clinical Procedures	68
10- Case Report Form	80
11- Approval form for Participation in Bioequivalence Study	90
12- Team of Investigators	96
Appendix I : C.V of the principal investigator and the PSC manager.	
Appendix II: Ethical Committee Approval Letter.	
Appendix III: Quality Management System ISO 9001-2000	

List of Tables

	<u>Page</u>
Table 1:	
<i>A:</i> Individual demographic and other baseline characteristics for volunteers participated in the study.	22
<i>B:</i> Blood analysis data for volunteers participated in the study.	23-24
Table 2: Individual data of % of baseline blood glucose concentration following a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 Human Insulin & Protamine – Insulin Human, SEDICO) to 24 healthy male volunteers.	25
Table 3: Individual data of % of baseline blood glucose concentration following a single subcutaneous injection (20 IU) of Mixtard 30 HM suspension (Biphasic Isophane Insulin injection 30/70, Novo Nordisk) to 24 healthy male volunteers.	26
Table 4: Pharmacodynamic parameters calculated after a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human, SEDICO) to 24 healthy male volunteers.	54
Table 5: Pharmacodynamic parameters calculated after a single subcutaneous injection (20 IU) of Mixtard 30 HM suspension (Biphasic Isophane Insulin injection 30/70, Novo Nordisk) to 24 healthy male volunteers.	55
Table 6: Mean Pharmacodynamic parameters calculated after a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human, SEDICO) or single subcutaneous injection (20 IU) Mixtard 30 HM suspension (Biphasic Isophane Insulin injection 30/70, Novo Nordisk) to 24 healthy male volunteers.	56
Table7: Bioequivalence determination of mean maximum reduction of blood glucose concentration following a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 Human Insulin & Protamine – Insulin Human) and Mixtard 30 HM suspension (Biphasic Isophane Insulin 30/70) as standardized using 90% confidence limits.	58

Table 8: Bioequivalence determination of mean time corresponding to 50% of the maximum reduction in the blood glucose following a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 Human Insulin & Protamine – Insulin Human) and Mixtard 30 HM suspension (Biphasic Isophane Insulin 30/70) as standardized using 90% confidence limits. **59**

Table 9: Bioequivalence determination of mean reduction of blood glucose concentration at 120 minutes following a single subcutaneous injection (20 IU) administration of Insulin H Mix 100 IU suspension (30/70 Human Insulin & Protamine – Insulin Human) and Mixtard 30 HM suspension (Biphasic Isophane Insulin 30/70) as standardized using 90% confidence limits. **60**

Table 10: Bioequivalence determination of mean area above the % of baseline blood glucose concentration – time curve following a single subcutaneous injection of Insulin H Mix 100 IU suspension (30/70 Human Insulin & Protamine – Insulin Human) and Mixtard 30 HM suspension (Biphasic Isophane Insulin 30/70) as standardized using 90% confidence limits. **61**

List of Figures:**Page**

Figures 1-24: % of baseline blood glucose concentration following a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human) and (20 IU) of Mixtard 30 HM suspension (Biphasic Isophane Insulin 30/70). **27**

Figure 25: Mean % of baseline blood glucose concentration following a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human) and (20 IU) of Mixtard 30 HM suspension (Biphasic Isophane Insulin 30/70). **51**

INTRODUCTION

Active Ingredient:

30/70 mixture Human Insulin & Protamine – Insulin Human.

Pharmacology:

- Insulin decreases the blood glucose level, enhances digestion of glucose by tissues, stimulates lipogenesis, glycogenesis, synthesis of protein, reduces the rate of glucose production in liver.

Pharmacokinetics:

- In case of subcutaneous injection, the drug begins to act in 30 minutes. Duration of the drug action is up to 24h. Profile of the drug action depends on dosage and scheme of individual therapy.

Therapeutic Uses:

- Diabetes mellitus type I (insulin-dependent) in children and adults; Diabetes mellitus type II (non-insulin dependent): at a stage of resistance to oral hypoglycemic drugs. Particular resistance to these drugs (combined therapy), intercurrent diseases, surgical operations (mono – or combined therapy); Diabetes mellitus type II in pregnant women.

Adverse Effects:

- Influence on carbohydrate metabolism: hypoglycemic state (paleness of dermal integuments, intensifying of diaphoresis, palpitation, tremor).
- Allergic responses: very rarely dermal eruption.
- Side reactions: lipodystrophy in places of drug administration (upon long term use).

- Others: temporal refraction disturbances (usually in the beginning of therapy).

Drug Interaction:

- Hypoglycemic effect of Insulin is enhanced by MAO inhibitors, some selective beta – blockers, sulfanilamide preparations, anabolic steroids, antibiotics of tetracycline group, clofibrate, cyclophosphamide, phenfluramine, ethanol-containing drugs. Hypoglycemic effect of Insulin is reduced by oral contraceptives, glucocorticoids, thyroid hormones, thiazide diuretic, heparin, lithium-containing preparations, tricyclic antidepressants.

Contraindications:

- Hypoglycemia, hypersensitivity to human insulin.

Objectives

This study was carried out to determine whether Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human, SEDICO), is bioequivalent to the commercially available Mixtard 30 HM suspension (Biphasic Isophane Insulin injection 30/70, Novo Nordisk) by comparing the blood glucose concentration at the indicated time points after administration of a single subcutaneous injection of each product.

STUDY DESIGN

Rationale of study design and justification of the study:

In vivo study:

The blood glucose concentration time profiles after administration of the two products in 24 normal healthy male volunteers were obtained from a two way cross over study with five days washout period between treatments.

STUDY MEDICATION

Test Drug

Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human), batch no. 0503101, manufacturing date 05/2003 and expiration date 11/2005, produced by SEDICO Pharmaceutical Co., 6 October City-Egypt.

Reference Drug

Mixtard 30 HM suspension (Biphasic Isophane Insulin injection 30/70), batch no. NS60194, manufacturing date 11/2002 and expiration date 04/2005, manufactured by Novo Nordisk A/S, 2880 Bagsvaerd, Denmark.

Study Protocol:

Site and duration of the study:

This study was conducted at the Pharmaceutical Services Center, College of Pharmacy, University of Tanta, Tanta, Egypt in collaboration with the staff members of Tanta University Hospital.

Selection of volunteers:

Twenty-four young adult male volunteers were chosen to participate in the present study. They were all within the normal weight range. The nature of the study was explained to all participants and written consents were obtained (Table 1A). All volunteers were examined clinically by a qualified attending physician and were pronounced fit to participate. The clinical assessment was complemented by laboratory examination (Table 1B), which confirmed that all the volunteers had hematological values, renal and hepatic functions within the normal ranges and all of them were non-diabetic. Exclusion criteria included diabetes, extreme weight ranges (overweight or underweight), anemia, liver or renal dysfunction, or thyroid dysfunction, parasitic, and other diseases that will affect the judgment on insulin efficacy.

The subjects were asked to abstain from taking drugs and alcohol for at least 3 days prior to the experiment and throughout the study period. On the night before the experiment, the volunteers were instructed to fast for at least 10 hours before drug administration.

Administration of the study drugs:

The volunteers were arbitrarily divided into two equal groups each of 12 subjects. The first group was given the reference preparation and the second group was given the test preparation with a crossover after a washout period of five days.

On the morning of the experiment, three blood samples were drawn from each volunteer at **(-30,-15,0 min)** to serve as the baseline blood

glucose concentration. Each of the 24 volunteers then took one subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human), as the test drug, or one subcutaneous injection (20 IU) of Mixtard 30 HM suspension (Biphasic Isophane Insulin injection 30/70), as the reference drug.

Dietary restriction:

Volunteers were not allowed to eat, drink juices or alcoholic beverages or smoke. They keep fasting throughout the four hours of the study, they were only allowed to drink water.

Collection of blood samples:

Blood samples were collected from finger tips using the lancing device to prick the side of finger tip. Samples were obtained at 0, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes after drug administration, the drop of blood is applied to the Accu-Chek Active monitor of Roche Diagnostics GmbH.

ANALYSIS OF BLOOD GLUCOSE CONCENTRATION

Principles of the assay:

A photometric method was used for blood glucose concentration determination.

Assay Conditions:

- An Accu-Chek Active monitor of Roche Diagnostics GmbH was used.
- The Accu-Chek Active strips were inserted in the monitor to which the blood drop is applied.
- The Accu-Chek Active strips differ from one pack to another so there is a coding chip that should be inserted into the slot on the side of the monitor before inserting the strips.
- Inserting the test strip automatically puts the monitor in test mode and applying the blood drop starts a colour reaction. The final colour is accurately read by the monitor's optical system and the result is converted to a blood glucose value in mg /dl.

Sample preparation:

- The finger tip is massaged to stimulate the circulation and make blood collection easier.
- The lancing device is used to prick the side of the massaged finger tip.
- A small drop of blood is formed.
- The drop of blood is applied to the middle of the orange, coloured square of the strip after flashing drop symbol appears.
- The results appear in the display, after 5 seconds

PHARMACODYNAMIC CALCULATIONS

The concentrations of glucose in the blood at different time points following administration of either single subcutaneous injection (20 IU) of Insulin H Mix 100 IU or single subcutaneous injection (20 IU) of Mixtard 30 HM were plotted for each individual volunteer (Figures 1 - 24). The blood glucose concentration were detected during the study. A baseline for blood glucose concentration was determined and the data of blood glucose concentration was calculated as a percentage (%) of baseline.

- ***Maximum Reduction in the blood glucose concentration:*** is the percentage (%) of lowest blood glucose concentration subtracted from the blood glucose concentration at baseline.

This parameter is used because it reflects the extent (intensity) of the therapeutic effect of insulin.

- ***Reduction of blood glucose concentration at 120 minutes:*** is the percentage (%) reduction of blood glucose concentration at 120 minutes which is the mid time of the study.

This parameter is used because it indicates the rate of production of the therapeutic effect.

- ***Time of the 50% maximum reduction in blood glucose concentration:*** is the time corresponding to 50% of the Maximum Reduction in the blood glucose.

This parameter is used because it indicates the rate of production of the therapeutic effect.

- **Area above the % of baseline blood glucose concentration – time curve:** was calculated from the blood glucose concentration using the relationship:

$$= \frac{(\% \text{ Reduction}_{t1} + \% \text{ Reduction}_{t2})}{2} \times (t2 - t1)$$

Where:

% Reduction_{t1} = percentage (%) of blood glucose concentration at time t1.

% Reduction_{t2} = percentage (%) of blood glucose concentration at time t2.

- This parameter is used because it reflects the extent of the therapeutic effect during the study period.

Statistical Analysis:

Assessment of Bioequivalence:

- The assessment of bioequivalence between the test and the reference products was based on the ratios of the log transformed of the mean pharmacodynamic parameters including the maximum reduction in blood glucose concentration, reduction of blood glucose concentration at 120 minutes, the time corresponding to 50% of the maximum reduction in the blood glucose and the area above the % of baseline blood glucose concentration – time curve.
- Bioequivalence was concluded if the 90% confidence limit of the difference between the log transformed mean values of each of the pharmacodynamic parameters for the test and reference products fall within the specified bioequivalence limit which is set at 80-120 % of the reference mean

IN VIVO BIOLOGICAL EVALUATION

Study population:

Twenty-four young adult (16-31 years, with a mean age of 21.50 ± 3.978 years) male volunteers were chosen to participate in the present study. They were all within the normal weight range (56-85 kg, with a mean weight of 68.87 ± 7.560 kg)

The demographic data for all the volunteers participated in this bioequivalence study is shown in (Table 1A).

Withdrawals:

The 24 volunteers, who enrolled, have completed the study, so no withdrawals.

Results of health state of the volunteers:

The following parameters were checked for all subjects participated in this study:

Clinical examination of cardiovascular, respiratory, renal, hepatic and gastrointestinal systems were performed. Also, all volunteers were subjected to complete examination to detect any psychiatric, neurological and genetic abnormalities, bleeding/coagulation disorders, severe anemia, thyroid dysfunction and infectious diseases. In addition vital signs were monitored before & during the study. This includes: blood pressure, pulse, respiratory rate and temperature. None of the participated subjects showed any abnormal signs concerning all the above checked parameters.

Monitoring of the adverse drug reactions:

All of the participated volunteers did not show any signs of adverse drug reactions except: Volunteer # 22 (during period I) showed mild dermal eruption. He did not need to receive any medication for that.

Biochemical results of the studied subjects:

The biochemical parameters of volunteers participated in the present study are shown in Table (1B). All volunteers had liver functions {Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT)} within the normal ranges. The volunteers also had normal kidney functions as indicated from their serum creatinine, and BUN. Furthermore, the blood glucose, cholesterol and triglycerides levels in all volunteers were found to be within the normal range. In summary all tests carried out before the study as well as the clinical examination confirmed that all of the volunteers were healthy and free from any apparent diseases.

% of baseline blood glucose concentration-time data:

% of baseline blood glucose concentrations at different time intervals following the subcutaneous injection of both products are summarized in Tables (2&3). The pharmacodynamic parameters determined for both products are summarized in Tables (4-6). The % of baseline blood glucose concentration time profiles following the administration of both products to the 24 volunteers are presented in Figures (1-24). The mean % of baseline blood glucose concentration time profile for both products in the 24 volunteers is shown in Figure (25).

Table (1A): Individual demographic and other baseline characteristics for volunteers participated in bioequivalence study of Insulin H Mix 100 IU (30/70 mixture Human Insulin & Protamine-Insulin Human) suspension.

Volunteer No.	Initials	Age (Years)	Weight (kg)	Height (Cm)	BMI	Systolic B.P (mmHg)	Diastolic B.P (mmHg)	Pulse (/ min)	Smoking
1	MME	19	73	174	24.11	120	80	77	NO
2	MMA	28	63	158	25.23	130	90	77	NO
3	AEA	20	63	166	22.86	120	90	75	NO
4	MMG	26	80	173	26.72	120	80	77	NO
5	TEA	23	56	165	20.56	130	80	76	NO
6	HES	23	64	168	22.67	120	70	75	NO
7	MAE	19	80	169	28.01	120	80	75	NO
8	MMS	16	73	170	25.25	130	90	77	NO
9	AAS	17	66	167	23.66	120	80	76	NO
10	AEG	20	63	163	23.71	120	80	76	NO
11	YZE	20	72	179	22.74	120	80	75	NO
12	MEE	19	63	166	22.86	110	70	75	NO
13	SAE	21	63	170	21.79	120	80	75	NO
14	MMH	21	68	177	21.70	130	90	77	NO
15	AFG	24	67	160	26.17	120	80	78	NO
16	MAB	23	83	174	27.41	120	80	77	NO
17	TME	21	71	182	21.43	120	70	77	NO
18	SMN	21	59	166	21.41	130	90	76	NO
19	AEE	16	69	173	23.05	120	80	75	NO
20	MEE	18	63	165	23.14	120	80	77	NO
21	HE	21	75	166	27.21	110	70	78	NO
22	SHE	19	65	172	21.97	120	80	78	NO
23	HES	31	85	183	25.38	130	90	77	NO
24	MAN	30	69	179	21.53	120	80	76	NO
Mean		21.50	68.87	170.2	23.77	121.6	80.83	76.33	
S.D.		3.978	7.560	6.587	2.195	5.646	6.538	1.049	
S.E.		0.812	1.543	1.344	0.440	1.152	1.152	0.214	

BMI: Body Mass Index

BP: Blood Pressure

Biochemical Study

Table (1B): Blood analysis data for volunteers participating in the study.

Volunteer No.	AST (U/L)	ALT (U/L)	Blood Urea (mg/dl)	Serum Creatinine (mg/dl)
Expected values	Up to 12	Up to 12	15-45	0.6-1.5
1	11	9.0	42	1.0
2	8.0	8.0	20	0.9
3	9.0	10	23	0.7
4	10	11	24	1.2
5	11	11	30	1.1
6	11	10	33	1.1
7	9.0	9.0	34	1.2
8	9.0	8.0	38	1.0
9	8.0	8.0	40	1.0
10	7.5	9.0	41	0.8
11	9.0	10	43	0.9
12	9.1	11	35	0.8
13	8.4	10	38	0.7
14	11	10	31	1.0
15	10	9.0	25	1.1
16	10	8.0	28	1.1
17	11	10	29	1.0
18	9.5	9.0	38	1.2
19	10	8.0	35	1.1
20	8.5	7.5	31	0.7
21	11	10	30	0.9
22	10	9.0	25	0.9
23	11	8.0	26	1.1
24	9.0	8.0	27	1.1
Mean	9.625	9.187	31.91	0.983
S.D.	0.878	1.091	6.526	0.157
S.E.	0.228	0.222	1.332	0.032

Table (1B): cont.

Volunteer No.	Fasting blood Glucose (mg/dl)	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	Uric Acid (mg/dl)
Expected values	75-115	Up to 250	Up to 165	Up to 7
1	92	175	150	5.1
2	83	200	100	5.5
3	107	210	70	4.0
4	88	230	110	4.5
5	99	180	130	6.0
6	93	190	140	6.3
7	83	230	155	6.5
8	79	245	160	5.2
9	86	240	90	4.3
10	97	235	80	5.5
11	77	180	95	6.1
12	107	190	100	6.3
13	91	195	101	6.5
14	81	188	88	5.5
15	94	178	99	4.3
16	96	199	100	4.7
17	98	190	110	6.2
18	90	210	120	3.6
19	80	220	130	3.7
20	81	215	150	5.0
21	81	230	145	5.2
22	95	240	130	5.3
23	93	210	120	5.5
24	83	220	100	6.0
Mean	89.75	208.3	115.5	5.283
S.D	8.522	22.05	25.30	0.878
S.E	1.439	4.502	5.165	0.179

Table (2): Individual data of % of baseline blood glucose concentration following a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 Human Insulin & Protamine – Insulin Human, SEDICO) to 24 healthy male volunteers.

Volunteer	Time (minutes)										
	0	15	30	45	60	90	120	150	180	210	240
1	100	100	94.25	89.65	85.05	77.01	74.71	59.77	63.21	72.41	81.60
2	100	95.29	89.41	80.00	72.94	64.70	89.41	65.88	62.35	62.35	63.52
3	100	84.61	87.50	83.65	77.88	71.15	66.34	64.42	67.30	68.26	62.50
4	100	98.73	94.93	88.60	88.60	88.60	88.60	86.07	86.07	83.54	92.40
5	100	98.92	93.54	83.87	76.34	67.74	63.44	61.29	63.44	79.57	82.79
6	100	96.55	97.70	95.40	89.65	75.86	72.41	74.71	72.41	68.96	65.51
7	100	97.56	96.34	91.46	91.46	90.24	89.02	81.70	86.58	73.17	69.51
8	100	101.1	90.58	90.58	81.17	83.52	78.82	81.17	75.29	90.58	89.41
9	100	95.12	103.6	96.34	96.34	97.56	91.46	89.02	90.24	73.17	80.48
10	100	84.61	80.76	75.96	71.15	75.96	72.11	65.38	63.46	64.42	72.11
11	100	97.70	93.10	94.25	82.75	82.75	82.75	77.01	75.86	71.26	70.11
12	100	97.67	91.86	79.06	69.76	75.58	79.07	75.58	73.25	80.23	73.25
13	100	101.0	84.78	78.26	75.00	64.13	63.04	69.56	69.56	72.82	75.00
14	100	102.1	95.74	77.65	60.63	47.87	58.51	52.12	52.12	57.44	63.83
15	100	106.9	76.74	77.90	67.44	47.67	51.16	53.48	58.14	61.62	66.27
16	100	106.8	107.9	80.68	72.72	76.13	68.18	69.31	70.45	72.72	79.54
17	100	97.95	92.85	91.83	77.55	68.36	60.20	61.22	65.30	64.28	68.36
18	100	79.76	67.85	72.61	83.33	75.00	63.09	54.76	66.66	65.47	77.38
19	100	96.34	102.4	100.0	89.02	92.68	78.04	81.70	86.58	79.26	85.36
20	100	82.10	85.26	82.10	60.00	67.36	71.57	72.63	73.68	70.52	73.68
21	100	98.85	96.55	102.2	89.65	86.20	86.20	81.60	81.60	87.35	88.50
22	100	100.0	88.29	81.91	74.46	79.78	80.85	77.65	76.59	76.59	77.65
23	100	92.30	84.61	71.42	67.03	56.04	57.14	58.24	70.33	70.33	61.53
24	100	96.62	95.50	96.62	86.51	80.89	74.15	83.14	80.89	79.77	78.65
Mean	100	96.20	91.34	85.92	78.60	74.70	73.34	70.72	72.14	72.75	74.96
SD	0	6.998	8.702	8.808	9.885	12.84	11.42	10.97	9.754	8.248	8.975
SE	0	1.428	1.776	1.798	2.018	2.623	2.332	2.241	1.991	1.684	1.832
MAX	100	106.9	107.9	102.2	96.34	97.56	91.46	89.02	90.24	90.58	92.40
MIN	100	79.76	67.85	71.42	60.00	47.67	51.16	52.12	52.12	57.44	61.53

Table (3): Individual data of % of baseline blood glucose concentration following a single subcutaneous injection (20 IU) of Mixtard 30 HM suspension (Biphasic Isophane Insulin 30/70 Novo Nordisk) to 24 healthy male volunteers.

Volunteer	Time (minutes)										
	0	15	30	45	60	90	120	150	180	210	240
1	100	95.65	90.21	88.04	90.21	83.69	79.34	72.82	63.04	94.56	104.3
2	100	106.0	100.0	83.13	77.10	66.26	69.88	66.26	67.47	61.44	68.67
3	100	90.65	83.17	71.96	65.42	55.14	62.61	70.09	69.15	67.28	59.81
4	100	94.31	101.1	101.1	92.04	87.50	82.95	78.40	84.09	80.68	80.68
5	100	94.94	95.95	90.90	83.83	71.71	58.58	47.47	72.72	124.2	95.96
6	100	100.0	81.72	82.79	77.41	74.19	72.04	73.11	66.66	60.21	68.81
7	100	98.79	102.4	92.77	87.95	84.33	86.74	83.13	83.13	71.08	81.92
8	100	94.93	92.40	81.01	82.27	79.74	75.94	72.15	68.35	70.88	68.35
9	100	98.83	95.34	96.51	97.67	87.20	87.20	73.25	74.41	77.90	72.09
10	100	86.59	87.62	82.47	87.62	75.25	69.07	60.82	57.73	57.73	65.97
11	100	92.20	85.71	74.02	76.62	72.72	67.53	59.74	64.93	68.83	70.13
12	100	99.06	78.50	65.42	75.70	86.91	73.83	78.50	64.48	69.15	57.94
13	100	94.50	86.81	79.12	74.72	58.24	59.34	63.73	65.93	68.13	70.32
14	100	101.2	93.82	86.41	75.30	61.72	55.55	59.25	55.55	58.02	62.96
15	100	106.3	93.61	88.29	72.34	82.97	79.78	62.76	68.08	59.57	53.19
16	100	101.0	96.87	87.50	82.29	79.16	86.45	69.79	89.58	75.00	72.91
17	100	87.75	88.77	78.57	73.46	75.51	67.34	70.40	66.32	68.36	72.44
18	100	88.88	78.88	75.55	74.44	77.77	57.77	58.88	55.55	53.33	48.88
19	100	100.0	100.0	91.25	82.50	87.50	85.00	80.00	72.50	81.25	73.75
20	100	100.0	97.53	90.12	83.95	80.24	72.84	77.77	79.01	77.77	74.07
21	100	97.53	98.76	101.2	93.82	100.0	88.88	82.71	80.24	85.18	83.95
22	100	91.57	75.78	71.57	70.52	62.10	68.42	75.78	63.15	72.63	73.68
23	100	81.72	80.64	73.11	64.51	61.29	61.29	65.59	59.14	63.44	64.51
24	100	98.79	92.77	83.13	83.13	78.31	69.87	63.85	75.90	77.10	77.10
Mean	100	95.89	90.77	84.00	80.20	76.23	72.43	69.43	69.46	72.66	71.77
SD	0	5.989	7.928	9.362	8.595	11.04	10.31	8.834	9.073	14.68	12.19
SE	0	1.228	1.618	1.911	1.754	2.254	2.106	1.803	1.852	2.998	2.490
MAX	100	106.3	102.4	101.2	97.67	100.0	88.88	83.13	89.58	124.2	104.3
MIN	100	81.72	75.78	65.42	64.51	55.14	55.55	47.47	55.55	53.33	48.88

Pharmacodynamic results:

- The maximum reduction in the blood glucose concentration following the administration of single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (SEDICO) ranged from 16.45 – 52.32 % of blood glucose concentration with a mean value of 34.06 ± 9.514 % of blood glucose concentration, whereas the maximum reduction in the blood glucose concentration following the administration of single subcutaneous injection (20 IU) of Mixtard 30 HM suspension (Novo Nordisk) ranged from 19.75 – 52.52 of blood glucose concentration, with a mean value of 36.86 ± 8.653 of blood glucose concentration. The time corresponding to 50% of the maximum reduction in the blood glucose was 57.50 ± 26.78 min after administration of single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension and 62.50 ± 25.66 min after administration of single subcutaneous injection (20 IU) of Mixtard 30 HM suspension. The reduction of blood glucose concentration at 120 minutes following the administration of single subcutaneous injection (20 IU) Insulin H Mix 100 IU suspension ranged from 8.537 – 48.83 % of blood glucose concentration with a mean value of 26.65 ± 11.42 % of blood glucose concentration whereas, the reduction of blood glucose concentration at 120 minutes following the administration of single subcutaneous injection (20 IU) of Mixtard 30 HM suspension (Novo Nordisk) ranged from 11.11 – 44.44% of blood glucose concentration, with a mean value of 27.56 ± 10.31 % of blood glucose concentration.

The area above the % of baseline blood glucose concentration – time curve after administration of single subcutaneous injection (20IU)

Insulin H Mix 100 IU suspension ranged from 2204 – 8752 % min blood glucose concentration, with a mean value of 5344 ± 1882 % min of blood glucose concentration, while following administration of single subcutaneous injection (20 IU) Mixtard 30 HM suspension, it ranged from 4842 – 9895 % min of blood glucose concentration, with a mean value of 7314 ± 1419 % min of blood glucose concentration.

- ***Assessment of bioequivalence:***

The insulin of Insulin H Mix 100 IU suspension (SEDICO) compared to the insulin of Mixtard 30 HM suspension (Novo Nordisk) was found to be bioequivalent.

It can be concluded that **Insulin H Mix 100 IU suspension** (30/70 mixture Human Insulin & Protamine Insulin Human, SEDICO) and **Mixtard 30 HM suspension** (Biphasic Isophane Insulin injection 30/70, Novo Nordisk) are bioequivalent, since both preparations produce equivalent therapeutic effect.

Table (4): Pharmacodynamic parameters calculated after a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human, SEDICO) to 24 healthy male volunteers.

Volunteer No.	Maximum reduction of blood glucose concentration (%)	Time of 50 % of maximum reduction in blood glucose concentration (min)	Reduction in blood glucose concentration at 120 minutes (%)	Area above the % baseline blood glucose concentration-time curve (%.min)
1	40.22	90	25.28	5439
2	37.64	45	10.58	6344
3	37.50	45	33.65	6598
4	16.45	30	11.39	2648
5	38.71	45	36.55	6080
6	34.48	60	27.58	4974
7	30.48	120	10.97	3246
8	24.70	90	21.17	3564
9	26.82	120	8.537	2204
10	36.53	90	27.88	6706
11	29.88	60	17.24	4370
12	30.23	45	20.93	5040
13	36.95	45	36.95	6301
14	52.12	45	41.48	8752
15	52.32	30	48.83	8651
16	31.81	45	31.81	5284
17	39.79	60	39.79	6658
18	45.23	15	36.90	7160
19	21.95	60	21.95	2936
20	40.00	45	28.42	6378
21	18.39	60	13.79	2749
22	25.53	30	19.14	4611
23	43.95	45	42.85	7730
24	25.84	60	25.84	3825
Mean	34.06	57.50	26.65	5344
S.D.	9.514	26.78	11.42	1882
S.E.	1.942	5.468	2.332	384.2
λ	52.32	120.0	48.83	8752
MIN	16.45	15.00	8.537	2204

Table (5): Pharmacodynamic parameters calculated after a single subcutaneous injection (20 IU) of Mixtard 30 HM suspension (Biphasic Isophane Insulin injection 30/70, Novo Nordisk) to 24 healthy male volunteers.

Volunteer No.	Maximum reduction of blood glucose concentration (%)	Time of 50 % of maximum reduction in blood glucose concentration (min)	Reduction in blood glucose concentration at 120 minutes (%)	Area above the % baseline blood glucose concentration-time curve (%.min)
1	36.95	90	20.65	6725
2	38.55	45	30.12	8159
3	44.85	30	37.38	7464
4	21.59	90	17.04	5531
5	52.52	90	41.41	7757
6	39.78	30	27.95	8056
7	28.91	90	13.25	5674
8	31.64	60	24.05	7424
9	27.90	90	12.79	5860
10	42.26	90	30.92	8675
11	40.26	60	32.46	8766
12	42.05	30	26.16	7752
13	41.75	45	40.65	7145
14	44.44	60	44.44	9499
15	46.80	60	20.21	7595
16	30.20	60	13.54	6242
17	33.67	45	32.65	8357
18	51.11	60	42.22	9624
19	27.50	90	15.00	6056
20	27.16	60	27.16	6583
21	19.75	120	11.11	4842
22	37.89	30	31.57	6710
23	40.86	30	38.71	9895
24	36.14	45	30.12	5150
Mean	36.86	62.50	27.56	7314
S.D.	8.653	25.66	10.31	1419
S.E.	1.766	5.239	2.106	289.8
<i>M</i>	52.52	120.0	44.44	9895
MIN	19.75	30.00	11.11	4842

Table (6): Mean Pharmacodynamic parameters calculated after a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin

Human, SEDICO) or single subcutaneous injection (20 IU) Mixtard 30 HM suspension (Biphasic Isophane Insulin injection 30/70, Novo Nordisk) to 24 healthy male volunteers.

PARAMETERS	Insulin H Mix 100 IU (SEDICO)	Mixtard 30 HM (Novo Nordisk)
Maximum reduction in % of baseline blood glucose concentration (%)	34.06 ± 9.514	36.86 ± 8.653
Time of 50% maximum reduction in blood glucose concentration (mins)	57.50 ± 26.78	62.50 ± 25.66
Reduction in blood glucose concentration at 120 minutes(%)	26.65 ± 11.42	27.56 ± 10.31
Area above the % baseline blood glucose concentration-time curve (%.min)	5344 ± 1882	7314 ± 1419

- Each parameter represents the mean ± S.D, n=24.
 - **Maximum reduction in the blood glucose concentration:** is the percentage (%) of lowest blood glucose concentration subtracted from the blood glucose concentration at baseline (presented as % of baseline).
 - **Reduction of blood glucose concentration at 120 minutes:** is the percentage (%) reduction of blood glucose concentration at 120 minutes which is the mid time of the study.

- **Time of the 50% maximum reduction in blood glucose concentration:** is the time corresponding to 50% of the maximum reduction in the blood glucose.
- **Area above the % of baseline blood glucose concentration – time curve:** was calculated from the blood glucose concentration using the relationship:

$$= \frac{(\% \text{ Reduction}_{t1} + \% \text{ Reduction}_{t2})}{2} \times (t2 - t1)$$

Where:

% Reduction t_1 = percentage (%) of blood glucose concentration at time t_1 .

% Reduction t_2 = percentage (%) of blood glucose concentration at time t_2 .

- **Statistical analysis of pharmacodynamic results:**

The 90% confidence limit of the difference between the log transformed mean values of maximum reduction in the blood glucose concentration, time corresponding to 50% of the maximum reduction in the blood glucose, reduction of blood glucose concentration at 120 minutes and area above the % of baseline blood glucose concentration – time curve for Insulin H Mix 100 IU and Mixtard 30 HM fall between (93.66-99.32%), (91.20-104.8%), (92.74-105.2%) and (82.67–89.27%) of the reference mean parameter values.(Tables 7-8-9&10). These confidence limits fall within the FDA specified bioequivalent limit (80-125%) indicating that the two products are bioequivalent.

Table (7): Bioequivalence determination of mean maximum reduction of blood glucose concentration following a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 Human Insulin & Protamine – Insulin Human) and Mixtard 30 HM suspension (Biphasic Isophane Insulin 30/70) as standardized using 90% confidence limits.

SUBJECTS	T	R	T-R	T/R	Log T/R	Exp
1	40.22	36.95	3.273	1.088573	0.036858	1.037545
2	37.64	38.55	-0.907	0.976475	-0.01034	0.989714
3	37.50	44.85	-7.359	0.835938	-0.07783	0.925125
4	16.45	21.59	-5.135	0.762169	-0.11795	0.888742
5	38.71	52.52	-13.81	0.736982	-0.13254	0.875865
6	34.48	39.78	-5.302	0.866734	-0.06211	0.939775
7	30.48	28.91	1.572	1.054364	0.022991	1.023257
8	24.70	31.64	-6.940	0.780699	-0.10752	0.898062
9	26.82	27.90	-1.078	0.961372	-0.01711	0.983037
10	36.53	42.26	-5.730	0.864436	-0.06327	0.938693
11	29.88	40.26	-10.37	0.7423	-0.12942	0.878604
12	30.23	42.05	-11.82	0.718865	-0.14335	0.866449
13	36.95	41.75	-4.801	0.885011	-0.05305	0.948332
14	52.12	44.44	7.683	1.172885	0.069255	1.07171
15	52.32	46.80	5.516	1.117853	0.048385	1.049575
16	31.81	30.20	1.610	1.053297	0.022551	1.022807
17	39.79	33.67	6.123	1.181837	0.072558	1.075255
18	45.23	51.11	-5.873	0.885093	-0.05301	0.94837
19	21.95	27.50	-5.549	0.798218	-0.09788	0.906759
20	40.00	27.16	12.84	1.472754	0.16813	1.183091
21	18.39	19.75	-1.362	0.931048	-0.03103	0.969449
22	25.53	37.89	-12.36	0.673759	-0.1715	0.842404
23	43.95	40.86	3.096	1.075771	0.03172	1.032228
24	25.84	36.14	-10.30	0.714981	-0.14571	0.864412
Mean						0.964969
S.D.						0.084226
Confidence limit						0.028279

The average ratio of maximum reduction of blood glucose concentration
(upper limit) = 0.993

The average ratio of maximum reduction of blood glucose concentration
(lower limit) = 0.936

This complies with the FDA limits (0.80-1.25)

Table (8): Bioequivalence determination of mean time corresponding to 50% of the maximum reduction in the blood glucose following a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 Human Insulin & Protamine – Insulin Human) and Mixtard 30 HM suspension (Biphasic Isophane Insulin 30/70) as standardized using 90% confidence limits.

Subjects	T	R	<i>T-R</i>	T/R	Log T/R	EXP
1	90	90	0	1	0	1
2	45	45	0	1	0	1
3	45	30	15	1.5	0.176091	1.192547
4	30	90	-60	0.333333	-0.47712	0.620567
5	45	90	-45	0.5	-0.30103	0.740056
6	60	30	30	2	0.30103	1.35125
7	120	90	60	1.666667	0.221849	1.133079
8	90	60	30	1.5	0.176091	1.192547
9	120	90	60	1.666667	0.221849	1.133079
10	90	90	0	1	0	1
11	60	60	0	1	0	1
12	45	30	180	7	0.845098	1.192547
13	45	45	0	1	0	1
14	45	60	-15	0.75	-0.12494	0.882551
15	30	60	-30	0.5	-0.30103	0.740056
16	45	60	-15	0.75	-0.12494	0.882551
17	60	45	15	1.333333	0.124939	1.133079
18	15	60	-45	0.25	-0.60206	0.547682
19	60	90	-30	0.666667	-0.17609	0.838541
20	45	60	-15	0.75	-0.12494	0.882551
21	60	120	-60	0.5	-0.30103	0.740056
22	30	30	0	1	0	1
23	45	30	15	1.5	0.176091	1.192547
24	60	45	15	1.333333	0.124939	1.133079
Mean						0.980349
S.D.						0.203445
Confidence limit						0.068308

The average ratio of time corresponding to 50% of the maximum reduction in the blood glucose (upper limit) =1.048

The average ratio of time corresponding to 50% of the maximum reduction in the blood glucose (lower limit) =0.912

This complies with the FDA limits (0.80-1.25)

Table (9): Bioequivalence determination of mean reduction of blood glucose concentration at 120 minutes following a single subcutaneous injection (20 IU) administration of Insulin H Mix 100 IU suspension (30/70 Human Insulin & Protamine – Insulin Human) and Mixtard 30 HM suspension (Biphasic Isophane Insulin 30/70) as standardized using 90% confidence limits.

SUBJECTS	T	R	T-R	T/R	Log T/R	Exp
1	25.28	20.65	4.635	1.22444	0.087938	1.09192
2	10.58	30.12	-19.53	0.351527	-0.45404	0.635057
3	33.65	37.38	-3.729	0.90024	-0.04564	0.955384
4	11.39	17.04	-5.653	0.668348	-0.175	0.83946
5	36.55	41.41	-4.855	0.882769	-0.05415	0.947287
6	27.58	27.95	-0.371	0.98673	-0.0058	0.994215
7	10.97	13.25	-2.277	0.82819	-0.08187	0.921392
8	21.17	24.05	-2.875	0.880462	-0.05529	0.946211
9	8.537	12.79	-4.254	0.667422	-0.1756	0.838954
10	27.88	30.92	-3.043	0.90161	-0.04498	0.956015
11	17.24	32.46	-15.22	0.531015	-0.27489	0.759653
12	20.93	26.16	-5.238	0.799832	-0.097	0.907555
13	36.95	40.65	-3.702	0.908931	-0.04147	0.959379
14	41.48	44.44	-2.955	0.933512	-0.02988	0.970562
15	48.83	20.21	28.62	2.416118	0.383118	1.466851
16	31.81	13.54	18.27	2.349579	0.37099	1.449169
17	39.79	32.65	7.143	1.218755	0.085916	1.089715
18	36.90	42.22	-5.317	0.87407	-0.05845	0.943222
19	21.95	15.00	6.951	1.4634	0.165363	1.179821
20	28.42	27.16	1.261	1.046429	0.01971	1.019905
21	13.79	11.11	2.682	1.241382	0.093906	1.098456
22	19.14	31.57	-12.43	0.606383	-0.21725	0.804726
23	42.85	38.71	4.147	1.10713	0.044199	1.04519
24	25.84	30.12	-4.277	0.857978	-0.06652	0.93564
Mean						0.989823
S.D.						0.185708
Confidence limit						0.062352

The average ratio of reduction of blood glucose concentration at 120 minutes (upper limit) = 1.052

The average ratio of reduction of blood glucose concentration at 120 minutes (lower limit) = 0.927

This complies with the FDA limits (0.80-1.25)

Table (10): Bioequivalence determination of mean area above the % of baseline blood glucose concentration – time curve following a single subcutaneous injection of Insulin H Mix 100 IU suspension (30/70 Human Insulin & Protamine – Insulin Human) and Mixtard 30 HM suspension (Biphasic Isophane Insulin 30/70) as standardized using 90% confidence limits.

SUBJECTS	T	R	T-R	T/R	Log T/R	Exp
1	5439.6	6725.5	-1285.8	0.808805	-0.09216	0.911963
2	6344.1	8159.6	-1815.5	0.777502	-0.1093	0.896463
3	6598.5	7464.9	-866.3	0.883938	-0.05358	0.947832
4	2648.7	5531.2	-2882.5	0.478867	-0.31979	0.726305
5	6080.6	7757.5	-1676.9	0.783831	-0.10578	0.899625
6	4974.1	8056.4	-3082.3	0.617406	-0.20943	0.811047
7	3246.9	5674.6	-2427.6	0.572189	-0.24246	0.784695
8	3564.7	7424.0	-3859.3	0.480158	-0.31862	0.727155
9	2204.2	5860.4	-3656.1	0.376126	-0.42467	0.653988
10	6706.7	8675.2	-1968.5	0.773088	-0.11177	0.894249
11	4370.7	8766.2	-4395.5	0.498583	-0.30226	0.739144
12	5040.6	7752.3	-2711.6	0.650216	-0.18694	0.829491
13	6301.6	7145.6	-843.9	0.881889	-0.05459	0.946877
14	8752.6	9499.9	-747.3	0.92133	-0.03558	0.965041
15	8651.1	7595.7	1055.3	1.138944	0.056503	1.058129
16	5284.0	6242.1	-958.1	0.846512	-0.07237	0.930189
17	6658.1	8357.1	-1698.9	0.796706	-0.0987	0.906013
18	7160.7	9624.9	-2464.2	0.743972	-0.12844	0.879463
19	2936.0	6056.2	-3120.2	0.484789	-0.31445	0.730192
20	6378.9	6583.3	-204.3	0.96896	-0.01369	0.986399
21	2749.9	4842.5	-2092.5	0.567878	-0.24575	0.782122
22	4611.7	6710.5	-2098.8	0.687234	-0.1629	0.84968
23	7730.7	9895.1	-2164.4	0.781262	-0.1072	0.898343
24	3825.8	5150.6	-1324.7	0.742795	-0.12913	0.878859
Mean						0.859719
S.D.						0.098276
Confidence limit						0.032996

The average ratio of area above the % of baseline blood glucose concentration – time curve (upper limit) = 0.892

The average ratio of area above the % of baseline blood glucose concentration – time curve (lower limit) = 0.826

This complies with the FDA limits (0.80-1.25)

DISCUSSION & SUMMARY

This report describes a study performed to determine the bioavailability of human insulin from Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human, SEDICO) relative to its bioavailability from Mixtard 30 HM suspension (Biphasic Isophane Insulin injection 30/70, Novo Nordisk). The later was taken as the reference drug. The study was conducted using a single subcutaneous injection (20 IU) of the test and reference in a 2-way cross over design using 24 healthy male volunteers. The criteria used to assess the bioequivalence of the two products were maximum reduction of blood glucose concentration, reduction in blood glucose concentration at 120 minutes, time of 50% maximum reduction in blood glucose concentration area above the % of baseline blood glucose concentration-time curve.

The mean maximum reduction of blood glucose concentration for insulin was found to be 34.06 ± 9.514 % and 36.86 ± 8.653 % following single injection of Insulin H Mix 100 IU (30/70 mixture Human Insulin & Protamine Insulin Human) and Mixtard 30 HM (Biphasic Isophane Insulin injection 30/70), respectively. While the mean time of 50% maximum reduction in blood glucose concentration for insulin was found to be 57.50 ± 26.78 min and 62.50 ± 25.66 min following the single injection of Insulin H Mix 100 IU (30/70 mixture Human Insulin & Protamine Insulin Human) and Mixtard 30 HM (Biphasic Isophane Insulin injection 30/70) respectively. The mean reduction in blood glucose concentration at 120 minutes for insulin was found to be 26.65 ± 11.42 % and 27.56 ± 10.31 following single injection of Insulin H Mix 100 IU (30/70 mixture Human Insulin & Protamine Insulin Human)

and Mixtard 30 HM (Biphasic Isophane Insulin injection 30/70) respectively. The mean area above the % of baseline blood glucose concentration-time curve for insulin was found to be 5344 ± 1882 %.min and 7314 ± 1419 %.min following single injection of Insulin H Mix 100 IU (30/70 mixture Human Insulin & Protamine Insulin Human) and Mixtard 30 HM (Biphasic Isophane Insulin injection 30/70) respectively.

Conclusion:

The assessment of bioequivalence between the test and the reference products was based on the ratios of the log transformed mean of the pharmacodynamic parameters (maximum reduction of blood glucose concentration, reduction in blood glucose concentration at 120 minutes, time of 50% maximum reduction in blood glucose concentration, area above the % of baseline blood glucose concentration-time curve). Bioequivalence was concluded if either tail probability did not exceed the 90% confidence limit and was completely contained in the 0.80 – 1.25 range.

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Clinical Protocol & Report

I-Introduction:

1. Source of Active Ingredients:

Insulin H Mix 100 IU suspension was supplied by SEDICO Pharmaceutical Co., 6 October City, Egypt.

2. Pharmacology:

- Insulin decreases the glucose level in blood, enhances digestion of glucose by tissues, stimulates lipogenesis, glycogenesis, synthesis of protein, reduces the rate of glucose production in liver.

3. Pharmacokinetics:

- In case of subcutaneous injection, the drug begins to act in 30 minutes. Duration of the drug action is up to 24h. Profile of the drug action depends on dosage and scheme of individual therapy.

4. Therapeutic Uses:

- Diabetes mellitus type I (insulin-dependent) in children and adults; Diabetes mellitus type II (non insulin dependent): at a stage of resistance to oral hypoglycemic drugs. Particular resistance to these drugs (combined therapy), intercurrent diseases, surgical operations (mono – or combined therapy); Diabetes mellitus type II in pregnant women.

5. Adverse Effects:

- Influence on carbohydrate metabolism: hypoglycemic state (paleness of dermal integuments, intensifying of diaphoresis, palpitation, tremor).
- Allergic responses: very rarely dermal eruption.
- Side reactions: lipodystrophy in places of drug administration (upon long term use).

II- Study Objectives:

The objective of the study was to compare the therapeutic effect of insulin from a test formulation; Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human) (SEDICO) in comparison with a reference formulation; Mixtard 30 HM (Biphasic Isophane Insulin injection 30/70) (Novo Nordisk). This was achieved by comparing the various pharmacodynamic parameters of insulin from the blood glucose concentration time profile of both formulations this including: maximum reduction of blood glucose concentration, reduction in blood glucose concentration at 120 minutes, time of 50% maximum reduction in blood glucose concentration, area above the % of baseline blood glucose concentration-time curve. To assess the bioequivalence of these two formulations the pharmacodynamic parameters were compared

1. Justification of the Study:

The use of generic preparation of therapeutically well-established active ingredients has to be justified by an appropriate bioequivalence study.

Evidence of bioequivalence of the test and the reference products is the most important to assure an equal therapeutic efficacy.

2. Risk to Benefit Ratio:

- Before the initiation of the trial, the risks and inconveniences are weighed against the anticipated benefits for the society.
- The safety, well-being of the trial subjects will be the most important consideration and will prevail over interests of science.
- The medical care given to subjects will be the responsibility of the qualified physician.

3. Treatment Doses:

The drug is administrated subcutaneously 1-2 times per day. Shake vial carefully before use. If the daily dose exceeds 0.6 IU/Kg, Insulin H Mix is injected 2 times into different parts of the body.

III- Investigations:

1. Location of the Study:

Pharmaceutical Service Center, College of Pharmacy, University of Tanta, Egypt. This center has established a quality management system that is in conformance with the International Quality System Standards (ISO 9001) from ASR in the scope of Bioequivalence studies. (Appendix III).

Analytical Facility:

The center is equipped with all the instruments required for drug analysis in the biological fluids including; Accu-Chek Active monitor. According to the international standards (ISO 9001), a regular and periodical calibration for all the equipments used for the analysis takes place.

Clinical Facility:

The clinical part of the bioequivalence studies is carried out in a separate clinic that is supervised by the staff members (Physicians & nursing staff) of Tanta University Hospital. This clinic is well equipped with housing facilities, instruments for medical examination & emergency measures and instruments used for collection of blood samples and biochemical analysis.

2. Laboratory Investigations:

Each volunteer is subjected to the following biochemical investigations before enrollment in the study.

- ◆ Liver function tests by measuring the serum level of AST and ALT.
- ◆ Kidney function tests by measuring the serum level of BUN, serum creatinine and uric acid.
- ◆ Lipid profile by measuring the serum level of cholesterol and triglycerides.
- ◆ Measuring blood glucose level.

The selected volunteers should have all their values to be within normal ranges.

IV- Method and Clinical Procedures:

1. Selection of Subjects:

1.1 Source of Subjects:

All the selected subjects are young adult male who live in Tanta city and its suburb areas. They are interested and willing to participate in the bioequivalence studies.

Selection of subjects will be based on:

The selected subjects must be male, their body weight-height ratio (BMI) must be within normal ranges. Medical history, vital signs, physical examination, biochemical, blood, urine examinations have to be within normal ranges. They have to sign the consent form.

1.2. Number of subjects:

24 adult male healthy volunteers.

1.3. *Informed consent form: (Regulatory affairs & Ethical considerations)*

The Ethical Committee of Tanta University Hospital guidelines will be followed with regard to the treatment of human volunteers in the study which meets the requirements of the declarations of Helsinki. The sponsor will be informed of the decision of the committee's decision. (Appendix II).

Before enrollment into the study, each volunteer will be given a complete explanation of the study including objectives, methods and possible risks. An informed consent form prepared by the investigators will be submitted to the ethical committee for approval. After assurance that all subjects understood the aim of the participation in the study, an informed consent form will be signed by the subject.

1.4. Subject Selection Criteria:

1.4.1 Inclusion Criteria:

Subjects should be healthy adult males with age between (16-31 years)
Subjects should have a normal body weight ($\pm 10\%$ of the ideal weight), a body

mass index (BMI) will be adopted and it equals to: weight (Kg) / height (m)², the results should be between 20- 30.

Subjects should understand the procedures and are willing to participate and gave their final written consent prior to the commencement of the study procedures. All demographic data and individual vital signs are presented.

1.4.2 Exclusion criteria:

- ◆ A known hypersensitivity to the drug.
- ◆ Thyroid dysfunction
- ◆ Diabetes.
- ◆ Gastrointestinal diseases.
- ◆ Autoimmune diseases.
- ◆ Renal diseases.
- ◆ Cardiovascular diseases.
- ◆ Pancreatic disease.
- ◆ Hepatic diseases (present or past history).
- ◆ Hematological disease, osteopathic or pulmonary diseases.
- ◆ Serious psychological illness.
- ◆ History of alcoholism or drug abuse.
- ◆ Serious psychological and neurological illness.
- ◆ Positive HIV.
- ◆ Abnormal laboratory values.
- ◆ Subjects who have taken any medication less than two weeks of the trials starting date.
- ◆ Subjects who have donated blood or who have been involved in multiple dosing study requiring a large volume of blood (more than 500 ml) to be drawn within 6 weeks preceding the start of the trials.
- ◆ Neoplastic diseases.

2. Study Design and Description:

2.1.Treatments Used in this Study:

Test Product:

Insulin H Mix 100 IU suspension.

Active Ingredient: (30/70 mixture Human Insulin & Protamine Insulin Human)

Batch Number: 0503101.

Manufacturing date: 05/2003

Expiration Date: 11/2005

Company: SEDICO Pharmaceutical Co., 6 October City, Egypt.

Country: Egypt.

Reference Product:

Mixtard 30 HM suspension.

Active Ingredient: (Biphasic Isophane Insulin injection 30/70)

Batch Number: NS60194

Manufacturing Date: 11/2002

Expiration Date: 04/2005

Company: Novo Nordisk A/S, 2880 Bagsvaerd, Denmark.

Country: Denmark.

2.2. Treatment Plan:

Assessment of bioequivalence between the test formulation (T) and the reference formulation (R) is carried out on 24 subjects. It follows a standard 2X2 cross-over design comprising two treatments.

The dosing period will be separated by a wash out period of sufficient length (at least 10 elimination half lives of the drug) for the drug received in the first period to be completely metabolized and / or excreted from the body and to ensure complete termination of the hypoglycemic effect of insulin and return of blood glucose levels to the normal values.

3-Clinical Procedures:

3.1. Physical & Laboratory Examination:

Subject's health status will be determined following a physical examination, laboratory tests and medical history by a qualified registered MD physician, Tanta University Hospital. The physician will review all preclinical laboratory tests for each subject. Tests will include the following:

Physical examination: Height, weight, blood pressure, heart rate, body temperature and respiratory rate

Blood chemistry: glucose, uric acid, BUN, creatinine AST, ALT, cholesterol, and triglycerides.

Hematological tests: Hemoglobin, Hematocrit, ESR, WBCs with differential, RBCs with platelets count and morphology, lymphocytes, MCV, MCH, monocytes, neutrophils, eosinophils, basophils, HIV and Hepatitis B screen.

Urine analysis: Specific gravity, pH and microscopic examination.

3.2. Randomization:

The volunteers were arbitrarily divided into two equal groups each of 12 subjects. The first group was given the reference preparation and the second group was given the test preparation with a crossover after a washout period of two weeks.

Randomization of volunteers (Design of the study):

Sequence	Subjects	Period I	Period II
1	1,2,3,4,5,6,7,8,9,10,11,12	R=1	T=2
2	13,14,15,16,17,18,19,20,21,22,23,24	T=2	R=1

T: Test Drug.

R: Reference Drug.

3.3. Administration of the Drugs:

Each volunteer received one subcutaneous injection (20 IU) of (30/70 mixture Human Insulin & Protamine Insulin Human) of Insulin H Mix 100 IU suspension (SEDICO) & Mixtard 30 HM suspension (Novo Nordisk) on two different occasions.

3.4. Times of administration:

All volunteers were admitted to study center at least 4 hours before drug administration which took place exactly at 10.00 AM.

3.5. Sample Collection:

Blood samples were collected from fingertips using the lancing device to prick the side of fingertips. Three samples were collected as control sample (15 min. in between) and then samples were obtained at 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes after drug administration.

Actual versus nominal blood sampling times (in minutes)

Time Points	0	15	30	45	60	90	
Normina	10.00	10.15	10.30	10.45	11.00	11.30	PM
	AM						

3.6. Diet:

No food or juice (nothing except water) was allowed during the study period.

3.7. Washout Period

Five days

3.8. Subjects Safety and Housing:

Subjects will report to the clinic by 6 A.M., where they will be housed for the duration of the study days. The study physician will be available throughout each study period. The subjects will be continuously observed during study period by the clinic staff and will report any adverse effects to the study physician. Safety of subject receiving the two products will be insured via the frequent monitoring of: Blood pressure, heart rate, body temperature and clinical observation of his general condition and all the vital signs. Gastrointestinal condition (GIT) including nausea, vomiting, diarrhea, constipation, or any other forms of GIT irritation must be also observed. Subjects should be asked for any signs of dermal eruptions, hypoglycemia or hypotension. Direct questions posed by the trial physician regarding adverse events and /or adverse drug reaction.

3.10. Analytical Procedure:

A photometric method used for blood glucose concentration determination.

3.12. Management of Inter Current Events:

Adverse drug reactions (ADR):

All noxious and unintended responses to the drug product related to the dose will be considered as adverse drug reactions. ADR will be monitored and recorded in each volunteers' CRF.

Adverse events (AE):

Any unfavorable signs including an: abnormal laboratory findings, symptoms, or diseases temporarily associated with the use of either of the investigational drug product, whether related or not related to the drug will be monitored and reported as an adverse events in the volunteer CRFs.

The following is regarded as specific adverse events for Insulin injection which will be observed at the specific time points:

Volunteers will be questioned at specific time points for ADR/AE:

- Palpitation.
- Tremor
- Paleness of dermal integuments
- Dermal eruption
- hypoglycemia

Serious Adverse Events:

The attending physician and staff members of the emergency department, Tanta University Hospital, located opposite to the center where the clinical trial is carried out. They be notified of the initiation and termination of the trial.

3.13. Reporting of Adverse Events or Serious Adverse Events:

Any adverse events and serious adverse events will be reported at the CRFs of each volunteer. The trial physician will be the contact person in cases of emergency.

3.14. Procedures to Ensure Proper Clinical Practice:

The clinical practice was monitored according the guidelines of GMP, GLP under supervision of PSC manager and principal investigator.

3.15. Concurrent Medication:

Subjects should be drug free for at least two weeks prior to study start date and through out the study. This include all prescription and/or over the counter preparation. Subjects will be reminded of this upon screening. No concomitant medication is permitted during the study. Subjects will be questioned on each study day prior to drug administration regarding this matter. Subjects will abstain from alcohol, or any other drinks or food that may interfere with the study.

3.16. Subject Withdrawal Criteria: (Premature Discontinuation)

Subjects will be withdrawn from the trial in any of the following instances:

- 1. Adverse drug reactions.**
- 2. Inter current illness.**
- 3. Non-compliance including:**
 - Consumption of food or juice (anything except water) during the study period.
 - Ingestion of any other medication (s) two weeks prior to the study or during the study.

- Socially unaccepted behavior, inconveniencing other participants

4. Subject's decision not to participate any further.

3.17. Modification of the Study Protocol:

Any modification of study protocol can be requested at any time during the actual investigation, which can be suggested and documented by the study physician or the principal investigator.

3.18. Variation from Clinical Protocol for the Individual Subject:

The study must be conducted in accordance with the protocol after approval, except in emergency situation. In such a situation, alteration of the protocol must be documented and explained.

3.19. Case Report Forms:

Case report forms including dosing & sampling time and results of medical, physical & laboratory investigations, ...etc will be supplied by the investigator. All the forms are to be completed. Subjects will be identified by initials and subject number.

Subjects with abnormal laboratory values, abnormal medical or physical result, etc will be excluded from the study. All medical data are to be reviewed by the study physician. Any adverse effects will be included in the case report records and summary reports. All the completed forms must be reviewed, signed dated by the physician & the principal investigator.

3.20. Subject Confidentiality

The confidentiality of records that could identify subjects will be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirements.

3.21. Monitoring, Quality Assurance & Audit of the Study:

The sponsor's monitor may visit the investigators and the study facility, in addition to maintaining necessary telephone and written communications, in order to maintain current knowledge of the study through review of the records comparison with source documents, observations, and discussion of the conduct of the study with

the investigators. In addition, the sponsor's audit may aim to evaluate the trial conduct and compliance with the protocol, SOP's, GCP and to assure that the systems are adequate to guarantee the quality and integrity of data supplied.

Because this center is **ISO 9001** certified, a regular monitoring, calibration of all used instruments, auditing is performed according to the international standards to assure application of the good laboratory practice. Quality control is applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

3.22. Drug Accountability:

Drug Inventory Records:

The principal investigator & the director of the study center must maintain an inventory record of drug received and dispensed.

Disposition of Unused Drug Supplies:

After completion of the study, all unused drug to be returned to the sponsor if this is requested. This include container of drug which were partially used and/or unopened supplies which were never dispensed.

3.23. Record Retention:

The investigator shall maintain the records of disposition of the drug and the records described above for two years following the completion of the study, unless is otherwise agreed upon by the sponsor.

3.24. Source Data and Documents:

All information in original records and certified copies of original records of clinical findings, observations or activities in the trial will be kept at the trial center.

The investigator will have a direct access to source data for all related monitoring, audits and quality assurance activities.

The sponsor will have access to CRF's without accessing the volunteer names or addresses.

3.25. Completion of the Study, Final Report:

Completion of the study:

All calculation should be performed under supervision of principal investigator; final report draft should be reviewed by PI & PSC director before its approval by PSC chairman of the board.

Final report:

- Two copies of original report will be submitted to the sponsor.
- 1) Data may be also provided on CD if this is requested by the sponsor, which will be enclosed with the original copy.
 - 2) A signed receipt of the final study will act as a signal for reconciling and finalizing the study.

Case Report Form

Test Product Name: Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human).

PSC Study Number:

Subject Number:

Subject Initials:

This page must be filed separately

1- Subject's Personal Data

- First name:.....
- Middle name:
- Family name:.....
- Street Address:.....
- City:
- Country:
- Telephone Number:
- Sex:
- Height:.....
- Weight:.....
- Date of Birth:
- Age: Years (at the time of the study)
- Age between 16 and 31 years? * Yes * No

If subject does not meet the respective selection criteria and the Principal Investigator decides that the subject can participate in the study it must be entered in a variations section, and a reason must be given.

Interviewer:

Date:

M.D. Name:

P.I.:

Case Report Form

Test Product Name: : Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human).

PSC Study Number:

Subject Number:

Subject Initials:

2- Medical History:

<i>Family history:</i>	* Normal	* Abnormal	
			Description
Allergy (including drug allergy):	* Normal	* Abnormal	
<i>In case of allergy to the test drug and/or related drugs, subject is <u>not</u> allowed to Participate.</i>			
Cardiovascular:	* Normal	* Abnormal	
Respiratory:	* Normal	* Abnormal	
Renal:	* Normal	* Abnormal	
Hepatic:	* Normal	* Abnormal	
Gastrointestinal:	* Normal	* Abnormal	
Surgery:	* No	* Yes	
Psychiatric diseases:	* No	* Yes	
Neurological diseases:	* No	* Yes	
Genetic abnormalities:	* No	* Yes	
Bleeding/coagulation disorders:	* No	* Yes	
Severe anemia:	* No	* Yes	
Infectious diseases:	* No	* Yes	
Others:	* No	* Yes	
Last blood Donation:		Date:	*No blood
Donation:			
Tobacco use: subject is smoker	* No	* Yes	
Alcohol use:	* No	* Yes	

Check whether subject meets all selection criteria, if subject does not meet the respective selection criteria, and the principal investigator decides that the subject can participate in the study it must be entered in a variations section, and a reason must be given.

Interviewer:

Date:

M.D. Name:

P.I.:

Case Report Form

Test Product Name: : Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human).

PSC Study Number:

Subject Number :

Subject Initials :

3- Physical Examination:

		Description
Appearance:	* Normal	*Abnormal
Skin:	* Normal	*Abnormal
Eye:	* Normal	*Abnormal
Nose:	* Normal	*Abnormal
Throat:	* Normal	*Abnormal
Mouth:	* Normal	*Abnormal
Lungs:	* Normal	*Abnormal
Heart:	* Normal	*Abnormal
Abdomen:	* Normal	*Abnormal
Kidney:	* Normal	*Abnormal
Lymph nodes:	* Normal	*Abnormal
Extremities:	* Normal	*Abnormal
Neurological and Reflex:	* Normal	*Abnormal
Mental Status:	* Normal	*Abnormal
Others:	*No	* Yes
If "Others", Please specify:		
.....		

Check, whether subject meets all selection criteria, if subject does not meet the respective selection criteria, and the principal investigator decides that the subject can participate in the study it must be entered in a variations section, and a reason must be given.

Interviewer:

Date:

M.D. Name:

P.I.:

<u>Case Report Form</u>		
Test Product Name: Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human)..		
PSC Study Number:	Subject Number:	Subject Initials:

4- Vital signs:

Study Day	Phase I (Day1)			Phase II (Day1)		
Date						
Actual Time (hh.mm)	9 am	12 pm	2 pm	9 am	12 pm	2 pm
Blood pressure (mm Hg)						
Pulse (/min)						
Respiration rate (/min)						
Temperature (°C)- oral						

Interviewer:

Date:

M.D. Name:

P.I.:

Case Report Form

Test Product Name: : Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human).

PSC Study Number:

Subject Number:

Subject Initials:

5- Laboratory Data:

- Biochemical Data: All values are within the normal , accepted ranges.

*Yes

* No

- Hematology Data: All values are within the normal , accepted ranges.

* Yes

* No

- Urinalysis Data: All values are within the normal , accepted ranges.

* Yes

* No

Interviewer:

Date:

M.D. Name:

P.I.:

Case Report Form

Test Product Name: : Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human).

PSC Study Number:

Subject Number:

Subject Initials:

6- Selection criteria:

		Description
• Male subject.	* Yes	*No
• Age between 16-45 years	* Yes	*No
• Height-weight range within the limits	* Yes	*No
• Medical history, vital signs, physical examination and laboratory test are without evidence of clinically significant medical condition.	* Yes	*No
• Biochemical & urine examination data are within normal ranges.	* Yes	*No
• Blood picture within the normal ranges	* Yes	*No
• Subjects consume alcohol.	* Yes	*No
• Subject takes drugs of abuse.	* Yes	*No
• Evidence for disorder in the metabolism Of drugs or any foreign compounds.	* Yes	*No

If subject does not meet the respective selection and the principal investigator decides that the subject can participate in the study it must be entered in a Variations section, and a reason must be given.

Interviewer:

Date:

M.D. Name:

P.I.:

Case Report Form

Test Product Name: Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human).

PSC Study Number:

Subject Number:

Subject Initials:

7- Clinical assessment:

Treatment A was well tolerated by the subject: *Yes *No
If No; Specify:

Treatment B was well tolerated by the subject: *Yes *No
If No; Specify:

Interviewer:

Date:

M.D. Name:

P.I.:

Case Report Form

Test Product Name: : Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human).

PSC Study Number:

Subject Number:

Subject Initials:

8- Safety assessment (ADR):

In Period (I):

* Adverse Drug Events:

.....
.....
.....

In Period (II):

* Adverse Drug Events:

.....
.....
.....

Interviewer:

Date:

M.D. Name:

P.I.:

Case Report Form

Test Product Name: : Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human).

PSC Study Number:

Subject Number:

Subject Initials:

9- Comments:

Any clinically important observation, not reported in other parts of the Case Report Form.

* Yes

*No

Comments	Date	Signature

Interviewer:

Date:

M.D. Name:

P.I.:

Case Report Form

Test Product Name: : Insulin H Mix 100 IU suspension (30/70 mixture

Human Insulin & Protamine Insulin Human).

PSC Study Number:

Subject Number:

Subject Initials:

10 - Signature of the Principal Investigator:

- I confirm that data included here is in complete accordance with the source documents of this subject.

Name of the Principal Investigator:

Signature:

Date:

Interviewer:

Date:

M.D. Name:

P.I.:

Approval Form for Participation of Volunteers in Bioequivalence Study

To be filled by the center employee:

Study Code:

Sponsor:

Pre – enrollment phase

Each selected volunteer has read all the following instructions and then signed the consent form which include the following:

- I have been informed by the research center that a comparative study for the plasma concentration of a pharmaceutical product with reference to a standard product to be conducted in fasting condition.

- ◆ Name of the active ingredient:

- ◆ Trade name of the test product:

Company:

- ◆ Trade name of the reference product:

Company:

- ◆ Therapeutic Category:

- ◆ Indications:

- I have been informed that I am one of at least 24 participants in this study.
- I accept to participate in the PSC bioequivalence study.
- I accept to undergo a medical check up by the study physician.
- I accept to have clinical laboratory investigations, which include blood chemistry & hematology analysis.
- I understand the objectives, procedures of the study withdrawal conditions, benefits, uses of the drugs & the possible side effects. All the parameters were actually and thoroughly explained to me by principal investigator.
The possible side effects may include the following:

1- More Frequent Side Effects:

.....

2- Less Frequent Side Effects:

.....

- I agree to stick with all the instructions given to me by the principal investigator.

Qualifications:

- I have no history of the following diseases: diabetes, asthma, gastric and duodenal ulcer, sinusitis, pharyngitis, renal or hepatic diseases, neurological diseases, cardiac or hematological diseases.
- My age is between 16-45 years old.
- I have not donated blood during the last three months.
- I don't need medical treatment and my health state does not require medical supervision.
- I am not allergic to any of the following drugs

Obligations & Commitments:

- I don't drink alcohol and I am not addict to any drug.
- I am willing to give the study center any blood or urine sample that will help the study

Signatures:

- | | |
|---|------------|
| • Name of the participant: | Signature: |
| • Name of the employee responsible for this from: | Signature: |
| • Name of the principal investigator: | Signature: |
| • Date: | |

Approval Form for Participation of Volunteers in Bioequivalence Study:

To be filled by the center employee:

Study Code:

Sponsor:

Enrollment phase (Information Concerning the Study)

Each participated volunteer has read all the following instructions and then signed the consent form which include the following:

- I have been informed that my medical examination and laboratory tests qualify me to participate in such study.
- Duration of the study is approximatelydays.
- I have to reach the study center at least hours before the time of dose administration of each phase.
- The study consists of two phases separated by a wash out period ofdays.
- I have to stay in the study center forhours after administering the dose of each phase.
- I accept to a single subcutaneous injection (20 IU) of Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human) (SEDICO) and a single subcutaneous injection (20 IU) of the reference drug Mixtard 30 HM suspension (Biphasic Isophane Insulin injection 30/70) (Novo Nordisk).
- I accept that blood samples (about one drop each) will be harvested at the following time points 0, 15, 30, 45, 60, 90, 120, 150, 180, 210 & 240 minutes after drug administration.
- I accept to let the attending physician to examine me and measure my heart rate, pulse and blood pressure before & during the study.
- I accept to give full details of any adverse effects that I may feel during or after the study to the physician.

Obligations & Commitments:

- I will not eat or drink anything except water during the study period.
- I have to avoid administering any non-prescription medication, except aspirin and vitamins, at least two weeks before the study.
- I have to avoid any drinks containing caffeine or alcohol at least 48 hours before the study.

Signatures:

- Name of the participant: Signature:
- Name of the employee responsible for this from: Signature:
- Name of the attending physician: Signature:
- Name of the principal investigator: Signature:
- Date:

Approval Form for Participation of Volunteers in Bioequivalence Study

To be filled by the center employee:

Study Code:

Sponsor:

Withdrawal from the study:

Each participated volunteer has read all the following instructions and then signed the consent form which include the following:

- I understand that I am free to withdraw from this study at any time without claiming any compensation.
- I understand that I may be requested to withdraw from this study, when I decide, with the physician and the investigator, that the decision was in my best interest. In this case, I will receive the full compensation.
- I understand that I may be requested to withdraw from this study at any time if I was found not complying with the investigator's instructions.

Benefits:

- The trial involves research and I am contributing to the advancement of knowledge in the area of drug research, studies and regulatory affairs for the benefit of all.
- I understand that there is no direct intended clinical benefit to me.
- I understand that I will receive a payment on completing the study according to the previous agreement with the principal investigator.
- I will sign a separate sheet on receiving the money.

Information:

- The volunteers may phone study center in the numbers.....for any information concerning the study or in emergency cases between the two phases.

Rights:

- I have the right to be treated under the expenses of the center in case I was sickened by any illness resulting from the study.

Signatures:

- Name of the participant: Signature:
- Name of the employee responsible for this from: Signature:
- Name of the attending physician: Signature:
- Name of the principal investigator: Signature:
- Date:

Team of Investigators in Bioequivalence Study of Insulin H Mix 100 IU suspension (30/70 mixture Human Insulin & Protamine Insulin Human) (SEDICO).

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Vice Dean, College of Pharmacy, University of Tanta.

2) Dr. Mohsen A. Hedaya, Ph. D.

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3) Dr. Wael Farrag, M.D.

Dept. of Internal Medicine, Tanta University Hospital.
Clinical Investigator.

4) Mr. Ahmed Goda, M.Sc. (Pharmacology)

Dept. of Pharmacology and Toxicology, College of Pharmacy, University of Tanta, Research Assistant.

5) Mrs. Noha El-Zawawy, B.Sc. (Pharmaceutical Sciences)

College of Pharmacy, University of Tanta.
Research Assistant (Analytical Monitor).

6) Dr. Ibrahim Hassan, Ph.D.

Professor of statistical analysis, College of Commerce, University of Tanta. Statistical Analysis Investigator.