


Pharmacokinetic and pharmacodynamic equivalence of Biocon's biosimilar Insulin 70/30 with US-licensed HUMULIN[®] 70/30 formulation in healthy subjects: Results from the RHINE-3 (Recombinant Human INsulin Equivalence-3) study

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Abstract

Aim: To establish the pharmacokinetic (PK) and pharmacodynamic (PD) equivalence of proposed biosimilar insulin 70/30 (Biocon's Insulin-70/30) and HUMULIN[®] 70/30 (HUMULIN-70/30; Eli Lilly and Company, IN).

Materials and Methods: In this phase 1, automated euglycaemic glucose clamp study, 78 healthy subjects were randomized (1:1) to receive a single dose of 0.4 IU/kg of Biocon's Insulin-70/30 and HUMULIN-70/30. Plasma insulin concentrations and glucose infusion rates (GIRs) were assessed over 24 hours. Primary PK endpoints were area under the insulin concentration-time curve from 0 to 24 hours - $AUC_{ins,0-24h}$ - and maximum insulin concentration - $C_{ins,max}$. Primary PD endpoints were area under the GIR time curve from 0 to 24 hours - $AUC_{GIR,0-24h}$ - and maximum GIR - GIR_{max} .

Results: Equivalence was shown between Biocon's Insulin-70/30 and HUMULIN-70/30 for the primary PK/PD endpoints. The 90% confidence intervals of the treatment ratios were entirely within the acceptance range of 80.00%-125.00%. The secondary PK/PD profiles were also comparable. There were no clinically relevant differences in the safety profiles of the two treatments and no serious adverse events were reported.

Conclusion: PK/PD equivalence was demonstrated between Biocon's Insulin-70/30 and HUMULIN-70/30 in healthy subjects. Treatment with Biocon's Insulin-70/30 and HUMULIN-70/30 was well tolerated.

KEYWORDS

basal-bolus insulin, biosimilar insulin, pharmacodynamics, pharmacokinetics, type 1 diabetes, type 2 diabetes

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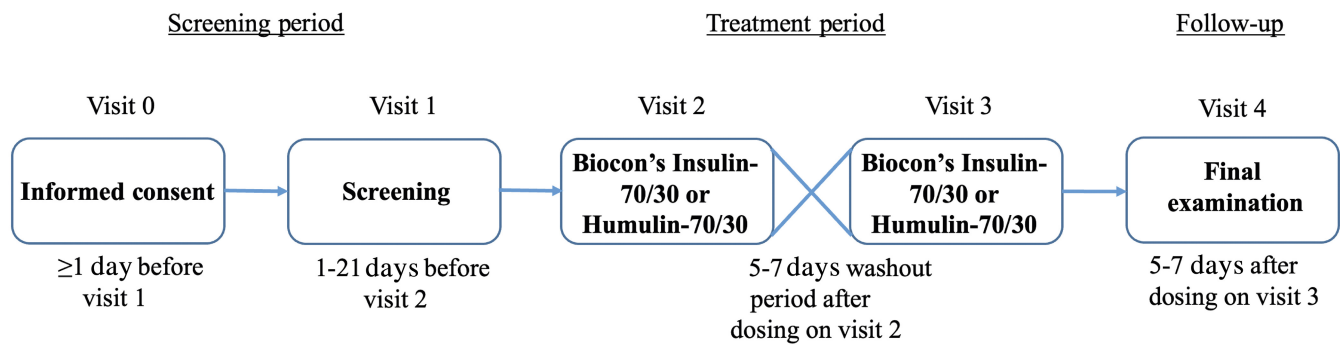


FIGURE 1 Study design

and all local and federal laws and regulations. The study was approved by a competent ethics committee (Ethikkommission der Ärztekammer Nordrhein in consultation with Ethik-Kommission der Landesärztekammer Rheinland-Pfalz) as well as a competent authority (Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM) before study initiation. Subjects provided written informed consent before initiation of any study procedure.

2.4 | Euglycaemic glucose clamping

Euglycaemic glucose clamping was performed using a glucose clamp device (ClampArt[®]; Profil Institut für Stoffwechselforschung, Neuss, Germany). The quality of the clamp data was reviewed regularly by the investigator and clamp supervisors, and evaluated based on all BG measurements during the clamp procedure²⁰ where the glucose infusion rate (GIR) was more than 0 mg/kg/min. The GIR necessary to keep the BG concentration at the target level was recorded every minute throughout the glucose clamp duration. BG was analysed at the study site using a Super GL glucose analyser (Dr. Müller Gerätebau GmbH, Freital, Germany) for verification of measurements of ClampArt. The mean clamp coefficient of variation (CV) was required to be less than 15% and the mean deviation from target (DFT) was required to be within the range of ±10 mg/dL after dosing until end of the clamp. Appendix S1 gives further details on the glucose clamp procedure.

2.5 | PK sampling

Blood samples were taken for PK (plasma insulin and serum C-peptide levels) analysis at predefined time intervals.

2.6 | Bioanalytical methods

Validated, ultra-performance, liquid chromatography with tandem mass spectrometry (LC-MS/MS) detection was used to analyse study samples. Insulin concentrations in plasma were measured using LC-MS/MS over a concentration range of 50-8000 ng/L. C-peptide in

serum was measured using a validated electrochemiluminescence immunoassay (Roche Diagnostics, Switzerland) over a concentration range of 0.2-32.0 ng/ml. All validation and sample quantification runs met prespecified acceptance criteria, including incurred sample reproducibility.

2.7 | PK assessment

Primary PK parameters included $AUC_{ins,0-24h}$ (area under insulin concentration-time curve from 0-24 hours) and $C_{ins,max}$ (maximum insulin concentration). Other parameters included $AUC_{ins,0-2h}$, $AUC_{ins,0-6h}$, $AUC_{ins,0-12h}$, $AUC_{ins,12-24h}$, $AUC_{ins,0-\infty}$ (AUC in indicated time intervals), $t_{ins,max}$ (time-to-maximum insulin concentration), $t_{50\%-ins(early)}$ (time-to-half-maximum insulin concentration before $C_{ins,max}$), $t_{50\%-ins(late)}$ (time-to-half-maximum insulin concentration after $C_{ins,max}$), $t_{1/2}$ (terminal elimination half-life) and λ_z (terminal elimination rate constant). The values of all individual PK parameters were calculated using non-compartmental methods in WinNonlin[®] v. 8.1 (Certara, Princeton, NJ). SAS[®] for Windows (SAS Institute Inc., NC; v. 9.4) was employed for all other statistical calculations ($t_{50\%-ins(early)}$, $t_{50\%-ins(late)}$, demography, PD parameters and safety) and all statistical analyses.

Primary PK analyses were conducted using Owen's method to correct endogenous insulin secretion by the C-peptide-based correction formula.²¹ Exogenous insulin concentration was calculated as:

$$\text{Insulin EXOG} = \text{Insulin OBS} - F * C - \text{peptide OBS},$$

where Insulin EXOG is exogenous insulin, Insulin OBS is observed insulin concentration, C-peptide OBS is observed serum C-peptide concentration and F is the mean of insulin/C-peptide concentration ratios at -30, -15 and 0 minutes.

A sensitivity analysis of the primary PK endpoints was performed using the same mixed model as described for the primary analysis with uncorrected (i.e. without applying Owen's correction for C-peptide) insulin concentrations. C-peptide-based exclusion rules (described in the next section for the primary PD parameters) were applied to the PK sensitivity analysis.

2.8 | PD assessment

Primary PD parameters included $AUC_{GIR,0-24h}$ (area under GIR time curve from 0-24 hours) and GIR_{max} (maximum GIR). Other parameters included $AUC_{GIR,0-2h}$, $AUC_{GIR,0-6h}$, $AUC_{GIR,0-12h}$, $AUC_{GIR,12-24h}$ (AUC_{GIR} in indicated time interval), $t_{GIR,max}$ (time-to-maximum GIR), $t_{50\%-GIR(early)}$ (time-to-half-maximum GIR before GIR_{max}), $t_{50\%-GIR(late)}$ (time-to-half-maximum GIR after GIR_{max}) and onset of action.

Primary PD analysis was conducted using C-peptide-based exclusion of profiles. For this, C-peptide concentration-time profiles were inspected during the blinded data review meeting to identify and exclude profiles as predefined for the study.

To account for meaningful fluctuations that can reflect changes in endogenous insulin concentration during the clamp period, profiles meeting predefined criteria were excluded from the primary PD analysis set. Sensitivity analysis for PD data was conducted using all profiles without applying any C-peptide-based exclusion rules.

2.9 | Safety assessments

All adverse events (AEs) were evaluated in terms of intensity, duration, severity, outcome and relationship to study medication throughout the study. Other safety parameters included injection site reactions, local tolerability, hypoglycaemic episodes, vital signs, physical examinations, 12-lead electrocardiograms (ECGs) and standard laboratory safety tests (Table S1).

2.10 | Statistical analysis

Equivalence between Biocon's Insulin-70/30 and HUMULIN-70/30 was considered shown if 90% confidence intervals (CIs) for primary PK endpoints, $AUC_{ins,0-24h}$ -ratio and $C_{ins,max}$ -ratio, and primary PD endpoints, $AUC_{GIR,0-24h}$ -ratio and GIR_{max} -ratio, lay within an acceptance interval of 80.00%-125.00%.

2.11 | Sample size

Based on a maximum conservative CV estimate of 35% and an assumed ratio of 0.95 between the reference and the test insulins, a sample size of 70 subjects was considered necessary to establish equivalence with sufficient power of at least 90% (sample size calculation based on $\alpha = 0.05$, 90% CIs in the range 80.00%-125.00%, ~10% dropout rate). Accounting for potential dropouts, a total of 78 subjects were randomized in the study.

2.12 | PK and PD endpoints

Analysis of PK and PD parameters was based on the respective per-protocol population (PPP). PPP for PK and PD included all randomized

subjects who completed the trial without any important protocol deviation. PK endpoints were derived from individual insulin concentration-time profiles and PD endpoints were derived from individual GIR profiles. Raw GIR and BG profiles were used to calculate GIR AUCs and onset of action. GIR_{max} and other time-related PD parameters were calculated from smoothed GIR profiles (SAS procedure PROC LOESS with smoothing factor 0.25). Single profiles of subjects who did not provide evaluable PK data were excluded from the PPP for PK if less than 50% of concentration measurements were above the lower limit of quantification or zero postdosing (i.e. 12/25 measurements). Single profiles of subjects who did not meet clamp quality criteria were excluded from the PPP for PD analysis.

The PK/PD endpoints (insulin and GIR AUCs, $C_{ins,max}$ and GIR_{max}) were analysed with analysis of variance (ANOVA) using log-transformed data. The ANOVA was based on a mixed model (proc MIXED) using clinical site, sequence, period, treatment, site by sequence and site by treatment as fixed effects and subject within site by sequence as a random effect. As site by treatment effect was not significant at the 5% level, this fixed effect was dropped from the model.

Within the model, the least square mean (LS-mean) for each treatment and difference of LS-means between the treatment groups and corresponding 90% CI were calculated, exponentially back-transformed, and multiplied by 100 to determine the estimated ratio % of responses between insulin formulations and corresponding 90% CIs.

2.13 | Safety

Analysis of safety endpoints was based on the safety analysis set, which included all randomized subjects who received at least one dose of study treatment. Safety data were summarized by visit/treatment using descriptive statistics.

3 | RESULTS

3.1 | Subject disposition and baseline characteristics

Of the 110 subjects screened, 78 (77 males and one female) were randomized to one of the two treatment sequences. Seventy-four subjects completed the study. Two subjects voluntarily withdrew from the study after treatment with HUMULIN-70/30 and two subjects were withdrawn by the investigator because of general discontinuation criteria (low-dose administration because of wrong body weight documentation; clamp termination because of massive recurring cannula problems) after treatment with Biocon's Insulin-70/30.

The age, BMI and FPG ranged from 19 to 55 (mean 37.1) years, 19.0 to 29.0 (mean 24.68) kg/m^2 and 59 to 100 (mean 87.7) mg/dL, respectively. Demographic characteristics were similar for both treatment sequences. No subjects received concomitant medication before

TABLE 2 Summary of treatment-emergent adverse events (TEAEs)

	Biocon's Insulin 70/30 (N = 76) m, n (%)	HUMULIN 70/30 (N = 77) m, n (%)
All TEAEs	21, 18 (23.7%)	22, 17 (22.1%)
Causally related TEAEs	7, 7 (9.2%)	9, 7 (9.1%)
TEAEs by severity		
Mild	18, 16 (21.1%)	16, 13 (16.9%)
Moderate	3, 3 (3.9%)	6, 6 (7.8%)
Severe	-	-
TEAEs by outcome		
Recovered/resolved	20, 17 (22.4%)	20, 15 (19.5%)
Recovering/resolving	1, 1 (1.3%)	2, 2 (2.6%)
TEAEs by SOC		
Gastrointestinal disorders	1, 1 (1.3%)	3, 3 (3.9%)
Abdominal pain upper	-	1, 1 (1.3%)
Diarrhoea	-	1, 1 (1.3%)
Nausea	1, 1 (1.3%)	1, 1 (1.3%)
General disorders and administration site conditions	3, 2 (2.6%)	3, 2 (2.6%)
Injection site reaction	1, 1 (1.3%)	2, 1 (1.3%)
Oedema	1, 1 (1.3%)	-
Oedema peripheral	-	1, 1 (1.3%)
Peripheral swelling	1, 1 (1.3%)	-
Infections and infestations	1, 1 (1.3%)	1, 1 (1.3%)
Gastroenteritis	-	1, 1 (1.3%)
Oral herpes	1, 1 (1.3%)	-
Injury, poisoning and procedural complications	2, 2 (2.6%)	6, 5 (6.5%)
Infusion site erythema	-	1, 1 (1.3%)
Infusion site haematoma	-	1, 1 (1.3%)
Infusion site pain	1, 1 (1.3%)	1, 1 (1.3%)
Infusion site swelling	1, 1 (1.3%)	1, 1 (1.3%)
Vascular access site inflammation	-	2, 2 (2.6%)
Musculoskeletal and connective tissue disorders	2, 2 (2.6%)	-
Pain in extremity	2, 2 (2.6%)	-
Nervous system disorders	8, 8 (10.5%)	7, 7 (9.1%)

(Continues)

TABLE 2 (Continued)

	Biocon's Insulin 70/30 (N = 76) m, n (%)	HUMULIN 70/30 (N = 77) m, n (%)
Headache	8, 8 (10.5%)	7, 7 (9.1%)
Respiratory, thoracic and mediastinal disorders	-	1, 1 (1.3%)
Oropharyngeal pain	-	1, 1 (1.3%)
Vascular disorders	4, 4 (5.3%)	1, 1 (1.3%)
Haematoma	3, 3 (3.9%)	1, 1 (1.3%)
Thrombophlebitis	1, 1 (1.3%)	-
Hypoglycaemic TEAEs		
Overall	14, 11 (14.5%)	21, 14 (18.2%)
Causally related hypoglycaemic TEAEs	11, 9 (11.8%)	18, 13 (16.9%)
Hypoglycaemic TEAEs by severity		
Mild	14, 11 (14.5%)	18, 14 (18.2%)
Moderate	-	3, 1 (1.3%)
Hypoglycaemic TEAEs by outcome		
Recovered/resolved	14, 11 (14.5%)	21, 14 (18.2%)
Clinically significant hypoglycaemia	-	3, 1 (1.3%)
Documented symptomatic hypoglycaemia	-	1, 1 (1.3%)
Asymptomatic hypoglycaemia	14, 11 (14.5%)	17, 13 (16.9%)

Note: N, number of patients in the relevant treatment group; m, number of events; n, number of patients; % = n/N *100.

Abbreviations: SOC, system organ class; TEAEs, treatment-emergent adverse events.

Results of sensitivity analysis, without applying any C-peptide-based exclusion rules, were similar to the primary analysis (AUC_{GIR,0-24h}: 90% CI 84.87%, 96.91%; GIR_{max}: 90% CI 86.56%, 99.08%; both within the 80.00%-125.00% limits), thus indicating the robustness of the study.

Mean values of secondary PD endpoints were overall comparable between Biocon's Insulin-70/30 and HUMULIN-70/30 (Table 1). Although secondary endpoints were not expected to meet the bioequivalence criteria, AUC_{GIR,0-6h}, AUC_{GIR,0-12h} and AUC_{GIR,12-24h} met the criteria.

3.2.3 | Clamp performance

No clamps were excluded based on predefined clamp quality criteria. Mean precision variability was less than 5% for both

treatments. Mean deviation from the clamp target was 0.142 and 0.216 mg/dL for Biocon's Insulin-70/30 and HUMULIN-70/30, respectively. Based on the fulfilment of acceptability criteria, the clamp quality was considered as good and comparable between the treatments.

3.3 | Safety

Overall, 43 treatment-emergent AEs (TEAEs; Biocon's Insulin-70/30: 21 TEAEs/18 subjects; HUMULIN-70/30: 22 TEAEs/17 subjects) were reported during the study (Table 2). The most frequently reported AEs were headache and haematoma. All TEAEs were 'resolved', except for three events that were 'resolving at follow-up visit' (Biocon's Insulin-70/30: moderate thrombophlebitis; HUMULIN-70/30: moderate infusion site pain and mild vascular access site inflammation), and all were considered unrelated to the study medication. The number of TEAEs was comparable between the two treatments (Biocon's Insulin-70/30: injection site reaction [$n = 1$], headache [$n = 6$]; HUMULIN-70/30: nausea [$n = 1$], injection site reaction [$n = 2$], headache [$n = 6$]). No serious AEs, deaths or discontinuations for safety/tolerability reasons occurred in the study. All the AEs were mild to moderate in intensity (Biocon's Insulin-70/30: mild 18, moderate three; HUMULIN-70/30: mild 16, moderate six).

After treatment with HUMULIN-70/30, three clinically significant hypoglycaemic events of moderate severity were observed in one subject (two related and one unlikely related) and one documented symptomatic hypoglycaemic event of mild severity was observed in another subject (deemed related). All events had the outcome of being 'resolved'.

No clinically significant changes in vital signs, physical examinations or ECGs were observed. There were no other clinically significant findings in haematology, biochemistry or urinalysis clinical laboratory tests throughout the study.

4 | DISCUSSION

This study showed similarity in rate and extent of absorption and glucose-lowering activity between Biocon's Insulin-70/30 and HUMULIN-70/30. Demonstration of PK/PD equivalence was based on 90% CI, for the ratio of test and reference products, being contained within the predefined acceptance limits of 80.00%-125.00%.² This was supplemented by similar results of the secondary endpoints.

A crossover, double-blind, euglycaemic clamp trial using single subcutaneous doses of test and reference formulations is considered most suitable by the FDA² and European Medicines Agency¹⁸ to simultaneously assess exposure and activity of biosimilar insulin products.^{2,18,22,23} The clamp setting, based on an automated glucose clamp technique with continuous BG measurements and minute-by-minute adaptations of GIRs, achieves the highest clamp quality possible and reduces potential investigator-related bias and

the risk of any drug-induced hypoglycaemia.^{24,25} The quality of clamp performance is critical for interpretation of the data.¹⁸ In this study, based on the fulfilment of acceptability criteria, the clamp quality (both precision and DFT data) was considered good and comparable between the treatments. A clamp duration of 24 hours, considered as a clinically meaningful treatment duration, was chosen to assess the complete single-dose PK/PD profile of both drugs.

In clinical settings, the treatment dose is individualized based on body weight, metabolic needs, BG monitoring results and glycaemic control goal; and the same was followed in this study. The study included healthy male and postmenopausal female subjects, representing the most sensitive and thus appropriate trial population. Besides better availability, healthy subjects are known to exhibit lower intra-individual variability. The study population was as per the inclusion and exclusion criteria specified (healthy males and postmenopausal females irrespective of race/ethnicity) in the protocol. However, the actual enrolment was determined by the availability of healthy volunteers at the study site, not excluding any race or gender per se. It is noted that in the healthy subjects, presence of endogenous insulin can potentially interfere with the PK/PD assessments. The following measures ensured suppression of endogenous insulin and/or minimized potential interference with the PK/PD results: (a) using 0.4 IU/kg dose, which is towards the higher end of the recommended range for insulin doses in clamp studies, (b) selecting a clamp target of 81 mg/dL \pm 10% to facilitate suppression of endogenous insulin while avoiding induction of hypoglycaemia/counter-regulatory hormones at the lowest end of the target range, (c) determining C-peptide levels in parallel with insulin concentrations to identify subjects whose endogenous insulin production potentially interfered with insulin PK/PD measurements and (d) using C-peptide-based correction methods for primary analyses of PK parameters and C-peptide-based exclusion rules for primary analyses of PD parameters to further rule out any impact of endogenous insulin on PK/PD outcomes.

Both Biocon's Insulin-70/30 and HUMULIN-70/30 were generally well tolerated with no clinically relevant safety issues. Headache was the most prevalent AE in this study, as has been commonly reported in numerous other glucose clamp studies.²⁴⁻²⁷ Clinically significant and documented symptomatic hypoglycaemic events that occurred during the glucose clamp procedure with HUMULIN-70/30 were transient and resolved with intravenous glucose infusion. No clinically relevant differences were observed in the safety profiles of both the drug formulations with regard to type, frequency and severity of AEs; local tolerability; vital signs; physical examination; ECG; and clinical laboratory results.

Diabetes management is a lifelong process and has a significant economic impact on healthcare systems.²⁸⁻³⁰ Rising costs make access to affordable insulin far more difficult for people with diabetes, especially mid- to low-income individuals, those on high deductible health plans, or those who are uninsured in countries like the United States. According to T1 International's 2018 survey with 1478 respondents from 90 countries, insulin rationing is widespread, with 18% (253/1408) of all respondents and nearly 26% (162/627) of the US respondents reportedly

having rationed insulin at least once in the previous year.³¹ A recent editorial in *Nature Biotechnology* talks about the rising costs and hence inequitable access to insulin that is forcing one in four patients with diabetes in the United States to ration their insulin.³²

Biocon's biosimilar Insulin-70/30 can provide reliable and affordable access to patients who are candidates for premixed-insulins. A biosimilar cost advantage may also enable more patients who currently use vials and syringes to opt for pens. Insulin pens (reusable or disposable) are associated with better patient compliance because of accurate dosing, lower episodes of hypoglycaemia and less injection site pain, etc., and hence better control of diabetes in the long term.³³⁻³⁵ A report on the coverage of insulin delivery devices by US-managed care organizations shows that both the prescribers and the patients have a perception of insurance coverage preferring the usage of vials over pens.³³ This is, however, not the case, as the majority of insurance plans cover pens and vials to the same extent.³³ HUMULIN-70/30 vials and pens have a common approved label from the FDA and the same would apply to Biocon's biosimilar Insulin-70/30. As such, vials and pens may be used interchangeably as per the requirement of the patient.

In conclusion, this study demonstrated equivalence between Biocon's Insulin-70/30 and HUMULIN-70/30, when administered as a single subcutaneous injection, for the primary PK/PD endpoints. The study also showed equivalence for the secondary PK/PD endpoints between the two treatments. Both insulin preparations were well tolerated and had similar safety profiles.

AUTHOR CONTRIBUTIONS

Design: SNA, SL, AM and SMNM. Conduct/data collection: OK, LPM, GS, NS and JP. Analysis: GS, SL, AM, SMNM and GCL. Writing and review of the manuscript: all the authors. All the authors read and approved the final version of the manuscript. SNA, as the guarantor of this work, takes full responsibility for the work, including the study design, access to data and the decision to submit and publish the manuscript.

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CONFLICT OF INTEREST

LPM has received speaker honoraria and travel grants from Eli Lilly, Gan & Lee Pharmaceuticals and Novo Nordisk. GS, AM, JP, SL and SNA are employees of Biocon Biologics Ltd and hold stocks in Biocon. SMNM and GCL are employees of Biocon Biologics Ltd. NS was employed with Biocon Biologics Ltd at the time of study conduct and preparation of the manuscript but is no longer employed by Biocon Biologics Ltd. OK is an employee of Profil, Neuss, Germany and has nothing to disclose.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/dom.14768>.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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