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ORIGINAL ARTICLE

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Pharmacokinetic and pharmacodynamic equivalence of Biocon's biosimilar insulin N with US-licensed Humulin® N formulation in healthy subjects: Results from the RHINE-2 (Recombinant Human INsulin Equivalence-2) study

Grit Andersen MD[1] | Gursharan Singh PhD[2] | Sundara Moorthi Nainar Murugesan PhD[2] | Rajesh Gogineni MPharm[2] | Nirant Sharma MSc[2] | Jayanti Panda MPharm[2] | Ashwani Marwah MSc[2] | Subramanian Loganathan MD[2] | Sandeep N. Athalye MD[2]

[1]Profil, Neuss, Germany

[2]Biocon Biologics Ltd., Bengaluru, India

Correspondence
Gursharan Singh, MBBS, Biocon Biologics Ltd., Biocon House, Tower 3, Semicon Park, Plot No 29-P1 & 31-P, KIADB Industrial Area, Electronic City Phase-2, Bangalore, 560100, Karnataka, India.
Email: gursharan.singh@biocon.com

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Abstract

Aim: To establish the pharmacokinetic (PK) and pharmacodynamic (PD) equivalence of proposed biosimilar Insulin N (Biocon's Insulin-N; Biocon Biologics Ltd., Bangalore, India) and US-licensed Humulin® N (Humulin-N; Eli Lilly and Company, Indianapolis, IN, USA) in healthy subjects.

Materials and Methods: This was a phase-1, single-centre, double-blind, randomized, three-period, six-sequence, partially replicated, crossover, 24-h euglycaemic clamp study. Overall, 90 healthy subjects were randomized, of whom 85 completed the study. The subjects received either two single doses of Biocon's Insulin-N and a single dose of Humulin-N or two single doses of Humulin-N and a single dose of Biocon's Insulin-N subcutaneously at a dose of 0.4 IU/kg. The primary PK endpoints were the area under the insulin concentration-time curve from 0 to 24 h ($AUC_{ins.0\text{-}24h}$) and the maximum insulin concentration ($C_{ins.max}$). The primary PD endpoints were the area under the glucose infusion rate (GIR) curve from 0 to 24 h ($AUC_{GIR.0\text{-}24h}$) and the maximum GIR (GIR_{max}).

Results: Biocon's Insulin-N was found to be equivalent to Humulin-N for the primary PK (geometric 90% confidence interval for the least squares mean ratio: $AUC_{ins.0\text{-}24h}$, 100.98%-115.66% and $C_{ins.max}$, 95.91%-110.16%) and PD endpoints (intra-subject variability ≥0.294; 95% upper confidence interval $[(\mu T - \mu R)^2 - \theta\sigma^2 WR] < 0$; point estimates of geometric least squares mean ratio: $AUC_{GIR.0\text{-}24h}$, 104.61% and GIR_{max}, 100.81%). The safety profile of Biocon's Insulin-N was similar to that of Humulin-N, and no serious adverse events were reported.

Conclusion: PK and PD equivalence was shown between Biocon's Insulin-N and Humulin-N in healthy subjects, and both treatments were well tolerated and considered safe.

KEYWORDS
bioequivalence, biosimilar, insulin N, NPH, partial replicate, pharmacodynamics, pharmacokinetics, RSABE, type 1 diabetes, type 2 diabetes

1 | INTRODUCTION

The direct annual cost of global health care expenditure because of diabetes is estimated to be US$ 980 billion[1] and, for many of the 537 million adults living now with diabetes, the cost of insulin is becoming prohibitive as its prices have tripled between 2002 and 2013.[2] About 100 million people have a critical need for insulin to maintain glycaemic control and prevent complications, comorbidities and subsequent mortality because of diabetes.[3,4] It is pertinent to note that the rising costs and hence inequitable access to life-saving insulin is forcing one in four patients, including in developed countries such as the United States, to ration their insulin,[5] as, for most of the under- and uninsured, the monthly costs of insulin can be about US$ 900.[5]

The WHO Steering Group strongly recommends the use of human insulin to manage blood glucose (BG) in adults with type 1 diabetes and for about 10% of patients with type 2 diabetes (T2D).[6–8] Recombinant human insulin (rHI) is the standard of care for diabetes management[9] and the regular, neutral protamine Hagedorn (NPH; isophane insulin) and premix insulins are economical alternatives to more expensive insulin analogs.[10] Moreover, introducing biosimilars of rHIs into the market will enhance the accessibility of economical insulin to patients in resource-constrained regions or situations. Recently, the Endocrine Society has made strong recommendations to expedite the approval of insulin biosimilars in the United States as one of the policies to increase access to life-saving insulin for patients with diabetes.[11]

Biocon's Insulin-N (Biocon Biologics Ltd., Bangalore, India), produced by *Pichia pastoris* (a yeast cell line) using recombinant DNA technology[12] has been developed as a proposed biosimilar to the US-approved Humulin® N (derived from *Escherichia coli*, a bacterial organism, hereafter referred to as Humulin-N; Eli Lilly and Company, Indianapolis, IN, USA). It is an intermediate-acting rHI that improves glycaemic control in adults and paediatric patients.[13] To ensure the similarity of the proposed product to the reference product, stringent regulatory requirements are met. These comprise numerous orthogonal analytical methods to evaluate the structural and functional similarity, further confirmed by pharmacokinetic (PK)/pharmacodynamic (PD) equivalence.[14] The presentation of similar PK/PD profiles is also considered proof of similar efficacy of the biosimilar and the reference insulin.[15]

This publication is the third in a series of clinical studies aimed at evaluating the PK/PD equivalence of Biocon's rHIs versus reference biologics in healthy subjects (RHINE studies). Results from RHINE-1 (Biocon's biosimilar Insulin-R vs. Humulin® R) and RHINE-3 (Biocon's biosimilar Insulin 70/30 vs. HUMULIN® 70/30) have been published earlier.[16,17] This study (RHINE-2) assesses the PK/PD equivalence and safety of Biocon's Insulin-N and Humulin-N using the euglycaemic clamp technique.

2 | MATERIALS AND METHODS

2.1 | Study design

This was a single-centre, randomized, double-blind, three-period, six-sequence, partially replicated, crossover, 24-h automated euglycaemic glucose clamp Phase 1 study (EudraCT: 2018-003216-44; Clinicaltrial.gov: NCT04022304) in healthy subjects. Subjects were randomized to one of six possible sequences and received either two single doses of Biocon's Insulin-N and a single dose of Humulin-N or two single doses of Humulin-N and a single dose of Biocon's Insulin-N, both 100 IU/ml, at a dose of 0.4 IU/kg body weight subcutaneously. Insulin was administered subcutaneously through a lifted skin fold of the abdominal wall into the peri-umbilical area using a standardized skin-fold technique. Insulin was administered at the left and right lower abdominal quadrants with a BD Micro-fine+0.5 ml U-100 syringe fitted with a 0.30 (30G) × 8 mm needle. The study design and randomization sequences are shown in Figure 1. All participants and staff, except those preparing the investigational medicinal product, were blinded until after study completion and the final blinded data review. Unblinded personnel preparing the investigational medicinal product did not participate in any other study activity. The subjects were dosed at the clinic after fasting for at least 10 h. During the 24-h euglycaemic clamp procedure, subjects remained fasted and water was allowed ad libitum. Blood samples were drawn for measuring insulin concentration and C-peptide levels (by-product of insulin secretion, widely accepted to reflect the extent/consistency of endogenous insulin suppression) as part of the PK/PD assessments. C-peptide levels were determined, in parallel, to identify subjects whose endogenous insulin production may potentially interfere with insulin PK and PD measurements, i.e. to identify variations of endogenous insulin secretion during the clamp procedure.[18] C-peptide-based correction methods were employed for primary analyses of PK and PD parameters to further rule out any impact of endogenous insulin on the PK or PD outcomes.

2.2 | Study subjects

Key inclusion criteria were healthy men and post-menopausal women aged 18-55 years (both inclusive), with body mass index 18.5-29.0 kg/m^2 (both inclusive) and fasting plasma glucose concentration ≤100 mg/dl. Key exclusion criteria were known or suspected hypersensitivity to either Biocon's Insulin-N or Humulin-N or any related products, having received any medicinal product in clinical development within 30 days or five times its half-life (whichever is longer) before randomization, and having a systolic blood pressure <95 mmHg or >140 mmHg and/or a diastolic blood pressure

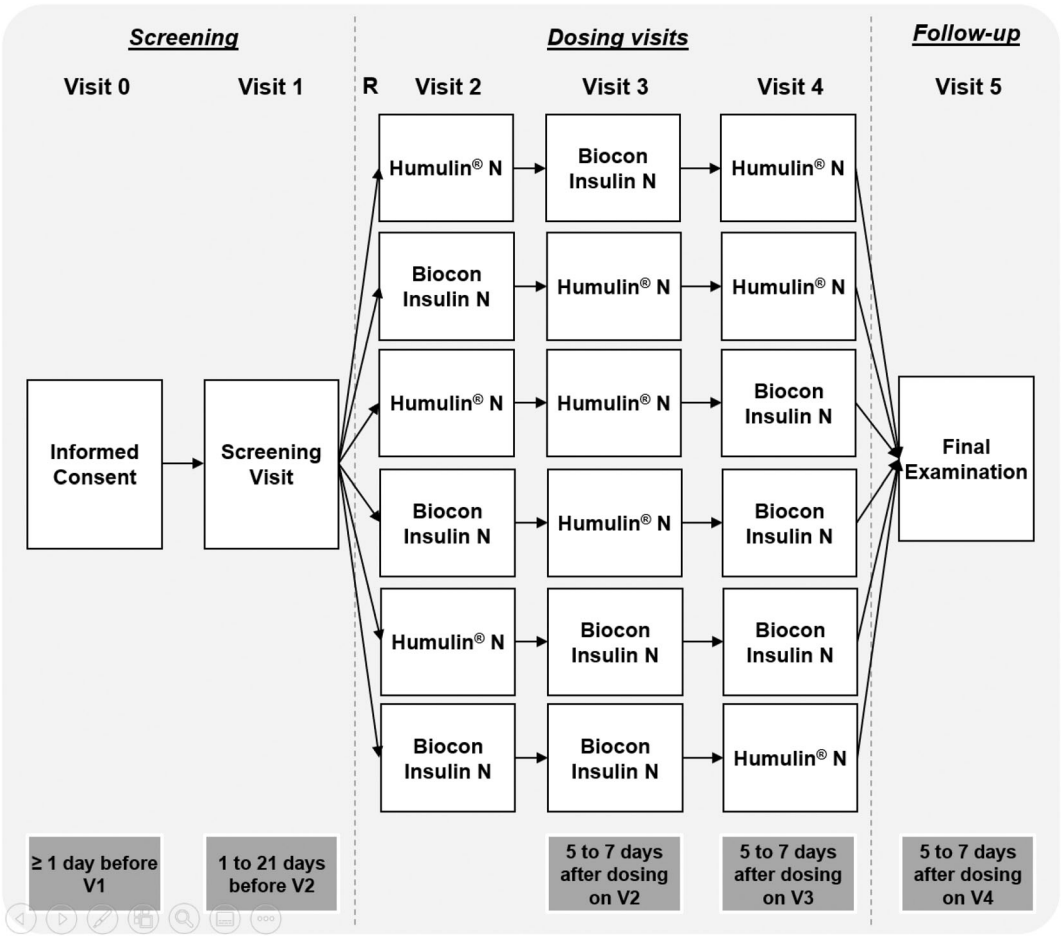


FIGURE 1 Study design

<50 mmHg or >90 mmHg or a pulse rate outside the range of 50-90 beats/min after resting for at least 5 min in the supine position.

2.3 | Ethics

Before the commencement of the study, all appropriate documents were approved by the ethics committee and/or Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM). The study was conducted in accordance with the ethical principles laid down in the International Council for Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki and all local and federal laws and regulations. All subjects provided written informed consent before study enrolment.

2.4 | Pharmacokinetic sampling

In total, 25 blood samples were collected for PK analysis from each subject at −30, −15 and 0 min (within −5 min of dosing) before dosing and at 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 480, 600, 720, 840, 960, 1080, 1200, 1320 and 1440 min post-dose.

2.5 | Euglycaemic glucose clamping

The automated euglycaemic glucose clamp was performed by a glucose clamp device (ClampArt®; Profil Institut für Stoffwechselforschung, Neuss, Germany). Following study treatment administration and a drop in the subject's BG by 5 mg/dl from baseline, the clamp device-controlled variable glucose infusion was initiated and BG was automatically kept at the target clamp level of 81 mg/dl. The glucose infusion rate (GIR) necessary to keep the BG concentration at the target level was recorded every minute throughout the glucose clamp. The glucose clamp lasted for 24 h after treatment administration. The investigators and clamp supervisors reviewed the quality of the clamp on a regular basis following the completion of each clamp. The quality of clamps was evaluated[19] on all BG measurements during the clamp procedure with GIR >0 mg/kg/min. The individual clamp coefficient of variation (CV; precision) was required to be <15% and the deviation from target (DFT; control deviation) was required to be within the range of ±10 mg/dl after dosing until the end of the clamp.

Apart from ClampArt®, the blood samples were analysed with a Super GL glucose analyser (Dr Müller Gerätebau GmbH, Freital, Germany) for verification of ClampArt® measurements (every 30 min or more frequently). Appendix S1 provides further details on the glucose clamp procedure.

2.6 | Bioanalytical methods

Insulin concentration in the plasma samples was determined using an automated immunoaffinity purification followed by ultra-performance liquid chromatography with tandem mass spectrometry detection. The lower limit of quantification was 50 ng/L and the upper limit of quantification was 8000 ng/L. The C-peptide in serum was measured using an electrochemiluminescence immunoassay (Roche Diagnostics), in which the lower limit of quantification was 0.2 ng/ml and the upper limit of quantification was 32.0 ng/ml. All validation and sample quantification runs fulfilled the predefined acceptance criteria.

2.7 | Pharmacokinetic assessments

Primary PK parameters included $AUC_{ins.0\text{-}24h}$ (area under the insulin concentration curve from 0 to 24 h) and $C_{ins.max}$ (maximum observed insulin concentration). Secondary PK parameters included $AUC_{ins.0\text{-}12h}$, $AUC_{ins.12\text{-}24h}$, $AUC_{ins.0\text{-}\infty}$ (areas under the insulin concentration curve during the time intervals), $t_{ins.max}$ (time to maximum observed insulin concentration), $t_{50\%\text{-}ins(early)}$ (time from dosing to the first time point where the concentration was $\geq C_{ins.max}/2$), $t_{50\%\text{-}ins(late)}$ (time from dosing to the first time point after $t_{ins.max}$ where the concentration was $\leq C_{ins.max}/2$), λ_z (terminal elimination rate constant of insulin) and $t_{½}$ (terminal elimination half-life).

2.8 | Pharmacodynamic assessments

Primary PD parameters included $AUC_{GIR.0\text{-}24h}$ (area under the GIR curve from 0 to 24 h) and GIR_{max} (maximum GIR). Secondary PD parameters included $AUC_{GIR.0\text{-}12h}$, $AUC_{GIR.12\text{-}24h}$ (areas under the GIR curve during the time intervals), $t_{GIR.max}$ (time to maximum GIR), $t_{50\%\text{-}GIR(early)}$ (time to half-maximum GIR before GIR_{max}), $t_{50\%\text{-}GIR(late)}$ (time to half-maximum GIR after GIR_{max}) and the onset of action.

2.9 | Safety assessments

Safety assessments included the recording of adverse events (AEs), including hypoglycaemic episodes, local tolerability/injection site reactions, vital signs, 12-lead electrocardiograms (ECGs), laboratory safety parameters (haematology, biochemistry and urinalysis) and physical examination (Table S1).

2.10 | Statistical analysis

2.10.1 | Pharmacokinetics and pharmacodynamics endpoints

All PK parameters [except $t_{50\%\text{-}ins(early)}$ and $t_{50\%\text{-}ins(late)}$] were calculated using standard non-compartmental methods using Certara WinNonlin® v8.1 (Certara, Princeton, NJ, USA) and the SAS® system for Windows v9.4 (SAS Institute Inc., Cary, NC, USA) statistical software for other statistical calculations and all analyses.

Analysis of PK and PD parameters was based on the respective per-protocol population (PPP), which included all randomized subjects of the full analysis set who received at least one dose of study treatment.

Primary PK/PD endpoints (insulin and GIR, AUCs, $C_{ins.max}$ and GIR_{max}) were analysed using logarithm-transformed data and the reference scaled average bioequivalence (RSABE) approach.[20]

Statistical analysis was performed using the RSABE approach. Bioequivalence between the trial insulins was shown if:

1. the intra-subject CV (ICV) of the reference product ≥30%, then the 95% upper confidence bound for $[(\mu T - \mu R)^2/\sigma^2 WR]$ is $\leq\theta$ or equivalently, a 95% upper confidence bound for $(\mu T - \mu R)^2 - \theta\sigma^2 WR$ is ≤0.00, and the point estimate (geometric mean ratio) falls within 0.80 and 1.25 inclusively OR
2. the ICV of reference product <30%, then the 90% confidence interval (CI) for the least squares mean ratios fall within the prespecified limits of 0.80 and 1.25 inclusively.

Where, μT = average of log-transformed parameter for the test treatment (Biocon's Insulin-N); μR = average of log-transformed parameter for the reference treatment (Humulin-N); $\sigma^2 WR$ = intra-subject variance of the reference treatment; $\theta = \log(\Delta)^2/\sigma^2 W0$ ($\Delta = 1.25, \sigma W0 = 0.25$).

PK/PD equivalence was shown if the 90% CI of the geometric LS-mean ratio of treatments fell within the limits of 80.00%-125.00%.

The secondary PK/PD endpoints were compared using the same statistical approach as for the primary PK/PD endpoints but were not required to fulfil the respective limits for equivalence.

While the RSABE approach was the primary statistical methodology for analysing primary PK/PD endpoints and for key secondary PK/PD endpoints, an additional statistical analysis of these PK endpoints was conducted using the standard average bioequivalence (SABE) approach. However, the results of the SABE approach were not considered for determining the formal pass or failure of the trial.

The primary PK analysis was conducted in a C-peptide-corrected dataset using Owen's method[21] for the correction of endogenous insulin secretion.

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

The primary PD analysis was conducted based on the following C-peptide exclusion rules: clamps with baseline C-peptide ≤0.5 nmol/L were excluded if the post-dosing C-peptide concentration increased to 1 nmol/L and clamps with baseline C-peptide >0.5 nmol/L were excluded if the post-dosing C-peptide concentration increased by at least 100% of the baseline value.

The sensitivity analysis of the primary PK endpoints was based on uncorrected data by applying C-peptide-based exclusion rules. However, the sensitivity analysis for primary PD endpoints was conducted by including all profiles in the PPP, without applying any C-peptide-based exclusion rules.

Time-related PK/PD endpoints were analysed using descriptive statistics by treatment/replicate only.

2.10.2 | Sample size

Considering an assumed ratio of 0.95 between test and reference insulin products, a sample size of 84 subjects in a partially replicated, six-sequence design[15,22] was needed to establish bioequivalence with sufficient power of at least 90% (sample size calculation based on $\alpha = 0.05$ and 90% CIs in the range of 80.00%-125.00%).

2.10.3 | Safety

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

3 | RESULTS

3.1 | Subject disposition and baseline characteristics

This euglycaemic study was successfully completed in 28 weeks (15 June-27 December 2019), with no protocol deviations impacting the statistical analyses. Of the 134 screened subjects, 90 were randomized to one of the six treatment sequences. Eighty-five subjects completed the study period. Five subjects discontinued the trial prematurely; of these, three subjects voluntarily withdrew consent while the other two subjects were withdrawn from the study by the investigator under the general discontinuation criteria. Of these five subjects, two were not dosed. All 90 subjects were included in the full analysis set and 88 subjects who received at least one dose of study treatment were included in the safety analysis set and the PPP.

Eighty-eight subjects were men (97.8%) and two subjects were post-menopausal women (2.2%). The age, body mass index and fasting plasma glucose ranged from 21 to 55 years (mean 40.0 years), 19.6-29.0 kg/m^2 (mean 25.1 kg/m^2) and 73-100 mg/dl (mean 90.1 mg/dl), respectively. The disposition, demographics and baseline characteristics of the subjects are presented in Table S2. Table S3 lists the medical history and concomitant illnesses.

3.2 | Pharmacology

3.2.1 | Pharmacokinetic analyses

The mean C-peptide-corrected plasma insulin concentration-time profiles (using Owen's method) were similar between Biocon's Insulin-N and Humulin-N (Figure 2). The intra-subject variability (s_{WR}) for both primary PK endpoints was <0.294. The SABE criterion (90% CI of the geometric LS-mean ratios within 80.00%-125.00%) was fulfilled for the primary PK endpoints, $AUC_{ins.0\text{-}24h}$ and $C_{ins.max}$ (Table 1).

Three profiles (all receiving Biocon's Insulin-N) were excluded from the sensitivity analysis of the primary PK endpoints. Results of sensitivity analysis, based on uncorrected data applying the C-peptide-based exclusion rules, confirmed those of the primary analysis (95% upper confidence limits: −0.0214 and −0.0368 and point estimates of the geometric LS-mean ratios: 103.52% and 99.95% for $AUC_{ins.0\text{-}24h}$ and $C_{ins.max}$, respectively).

Although secondary endpoints were not expected to meet bioequivalence criteria, $AUC_{ins.0\text{-}12h}$ was comparable for Biocon's Insulin-N and Humulin-N (Table 2).

3.2.2 | Pharmacodynamic endpoints

The primary analysis of PD data was conducted using C-peptide-based exclusion of profiles. The mean GIR profiles were similar between Biocon's Insulin-N and Humulin-N (Figure 3). Mean $AUC_{GIR.0\text{-}24h}$ and GIR_{max} were equivalent for both treatments. The RSABE model was used for the statistical analysis as the s_{WR} of Humulin-N was >0.294 (corresponding ICV = 30%) for both primary PD endpoints. The statistical comparison showed that $AUC_{GIR.0\text{-}24h}$ and GIR_{max} met both RSABE criteria (Table 1). The 95% upper confidence limits $[(\mu T - \mu R)^2 - \theta\sigma^2 WR]$ were −0.0662 and −0.0666 (<0) and the point estimates of the geometric LS-mean ratios were 104.61% and 100.81% for $AUC_{GIR.0\text{-}24h}$ and GIR_{max}, respectively (within 80.00%-125.00% limits).

The sensitivity analysis of the primary PD endpoints was made without applying C-peptide-based exclusion rules. Results confirmed those of the primary analysis (95% upper confidence limits: −0.0654 and −0.0664 and point estimates of the geometric LS-mean ratios: 105.14% and 101.17% for $AUC_{GIR.0\text{-}24h}$ and GIR_{max}, respectively).

The primary PD endpoints were also analysed using the SABE approach. All primary PD endpoints fulfilled the equivalence criterion (90% CI of the geometric LS-mean ratios within 80.00%-125.00% limits).

Although secondary endpoints were not expected to meet the bioequivalence criteria, $AUC_{GIR.0\text{-}12h}$ and $AUC_{GIR.12\text{-}24h}$ were overall equivalent for Biocon's Insulin-N and Humulin-N based on both the RSABE and the SABE approaches (Table 2).

3.2.3 | Clamp performance

The quality of clamps was evaluated based on all measurements during the clamp procedure where GIR was >0 mg/kg/min. The precision and DFT data showed that the clamp quality was good and comparable between treatments. Mean precision was <4% with both treatments. Mean DFT was −0.01 and 0.08 mg/dl after dosing with Biocon's Insulin-N and Humulin-N, respectively.

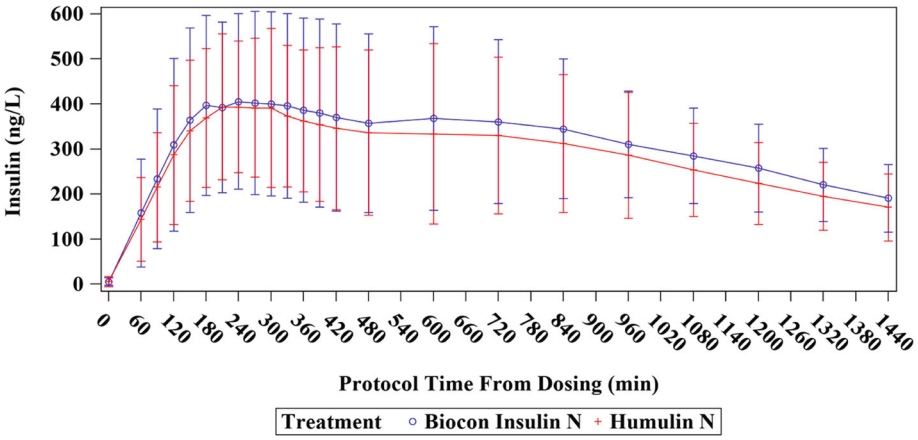


FIGURE 2 C-peptide corrected mean insulin profiles – linear scale (per-protocol population for pharmacokinetics)

TABLE 1 Primary PK and PD endpoints (PPP)

Summary statistic	PK endpoints - C-peptide corrected data (PPP for PK)		PD endpoints - based on C-peptide exclusion rules (PPP for PD)	
	$AUC_{ins.0\text{-}24h}$ (ng*h/L) (N = 87)	$C_{ins.max}$ (ng/L) (N = 87)	$AUC_{GIR.0\text{-}24h}$ (mg/kg) (N = 87)	GIR_{max} (mg/kg/min) (N = 87)
Geometric LS-mean: Form T [test = Biocon's insulin-N]	6932.397	481.167	2338.466	2.935
: Form R [ref = Humulin-N]	6414.501	468.120	2235.314	2.912
%ratio: T/R	108.07%	102.79%	104.61%	100.81%
90% CI: T/R	(100.98%; 115.66%)[a]	(95.91%; 110.16%)[a]	(96.85%; 113.00%)[b]	(94.13%; 107.96%)[b]
Intra-subject variability (s_{WR} ≥0.294; corresponding intra-subject CV = 30%): form T [test = Biocon's insulin-N][c]	0.3331	0.3045	0.3458	0.3074
: Form R [ref = Humulin-N][c]	0.2872	0.2921	0.3512	0.3399
95% upper confidence interval $(\mu T - \mu R)^2 - \theta\sigma^2 WR$	−0.0348	−0.0468	−0.0662	−0.0666

Abbreviations: ABE, average bioequivalence method; AUC, area under the curve; $AUC_{GIR.0\text{-}24h}$, area under the glucose infusion rate curve from 0 to 24 h; $AUC_{ins.0\text{-}24h}$, area under the insulin concentration curve from 0 to 24 h; CI, confidence interval; $C_{ins.max}$, maximum plasma insulin concentration; CV, coefficient of variation; GIR_{max}, maximum glucose infusion rate; LS, least squares; PD, pharmacodynamics; PK, pharmacokinetics; PPP, per-protocol population; RSABE, reference scaled average bioequivalence method.

[a]If the intra-subject variability in reference treatment <30% and the 90% CI of LS-mean ratio fell within 80.00% to 125.00%, PK bioequivalence was shown.

[b]If the intra-subject variability in reference treatment <30% and the 90% CI of LS-mean ratio fell within 80.00% to 125.00%, PD equivalence was shown.

[c]Had to follow progesterone guidelines, which had to be modified to fit a partial replicated three-way crossover with six sequences.

3.3 | Safety

Overall, 66 treatment-emergent AEs (TEAEs) were reported during the study (Table S4; Biocon's Insulin-N: 35 AEs/21 subjects, Humulin-N: 31 AEs/25 subjects). Most of the TEAEs were mild in severity (26 and 20 events with Biocon's Insulin-N and Humulin-N, respectively). The remaining AEs were moderate in severity (nine and 11 events with Biocon's Insulin-N and Humulin-N, respectively), and none were severe. The most common TEAE reported was headache (Biocon's Insulin-N: 16 events/12 subjects and Humulin-N: 13 events/12 subjects), followed by injection site reaction (Biocon's Insulin-N: one event/one subject and Humulin-N: four events/four subjects). The number of drug-related, non-hypoglycaemic AEs was comparable between the treatments [Biocon's Insulin-N: nausea (n = 1), oral discomfort (n = 1), injection site reaction (n = 1), headache (n = 7); Humulin-N: nausea (n = 1), vomiting (n = 2), injection site reaction (n = 4), headache (n = 8) and rash papular (n = 1)].

Overall, three treatment-emergent hypoglycaemic episodes (all mild and asymptomatic) in three subjects were reported during the clamp period. One hypoglycaemic episode occurred after dosing with Biocon's Insulin-N and was considered related and two hypoglycaemic episodes occurred after dosing with Humulin-N, of which one was considered definitely and the other probably related.

One clinically significant physical examination finding (hard changes of a vein, lower arm posterior right) was recorded during

TABLE 2 Secondary PK and PD endpoints (PPP)

Summary statistic	PK endpoints - C-peptide corrected data (PPP for PK)			PD endpoints - based on C-peptide exclusion rules (PPP for PD)	
	$AUC_{ins.0\text{-}12h}$ (ng*h/L) (N = 87)	$AUC_{ins.12\text{-}24h}$ (ng*h/L) (N = 87)	$AUC_{ins.0\text{-}\infty}$ (ng*h/L) (N = 87)	$AUC_{GIR.0\text{-}12h}$ (mg/kg) (N = 87)	$AUC_{GIR.12\text{-}24h}$ (mg/kg) (N = 87)
Geometric LS-mean: Form T [test = Biocon's insulin-N]	3657.943	3140.827	10 690.831	1317.100	955.865
: Form R [ref = Humulin-N]	3500.161	2835.203	9056.305	1302.822	876.562
%ratio: T/R	104.51%	110.78%	118.05%	101.10%	109.05%
90% CI: T/R	(96.59%; 113.07%)[a]	(102.41%; 119.83%)[a]	(108.28%; 128.70%)[a]	(92.51%; 110.47%)[b]	(98.75%; 120.42%)[b]
Intra-subject variability (s_{WR} ≥0.294; corresponding intra-subject CV = 30%): Form T [test = Biocon's insulin-N][c]	.3941	0.3040	0.1951	0.4308	0.3451
: Form R [ref = Humulin-N][c]	0.3509	0.2807	0.1995	0.4047	0.4493
95% upper confidence interval $(\mu T - \mu R)^2 - \theta\sigma^2 WR$	−0.0661	−0.0217	0.0207	−0.0941	−0.0989

Note: Secondary PK and PD AUC endpoints did not need to fulfil the respective limits for equivalence and were not considered for determining formal pass or fail of the study.

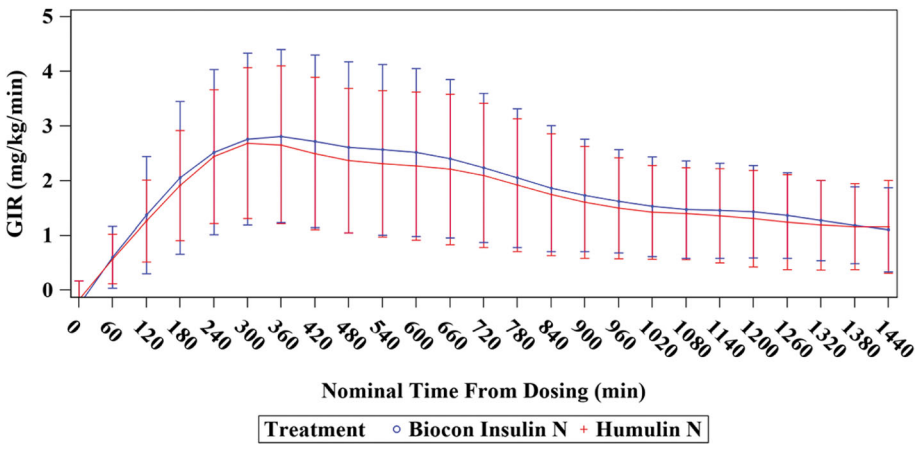
Abbreviations: ABE, average bioequivalence method; AUC, area under the curve; $AUC_{GIR.0\text{-}12h}$, area under the glucose infusion rate curve from 0 to 12 h; $AUC_{GIR.12\text{-}24h}$, area under the glucose infusion rate curve from 12 to 24 h; $AUC_{ins.0\text{-}12h}$, area under the insulin concentration curve from 0 to 12 h; $AUC_{ins.12\text{-}24h}$, area under the insulin concentration curve from 12 to 24 h; $AUC_{ins.0\text{-}\infty}$, area under the insulin concentration curve from 0 to ∞; CI, confidence interval; CV, coefficient of variation; LS, least squares; PD, pharmacodynamic; PK, pharmacokinetic; PPP, per-protocol population; RSABE, reference scaled average bioequivalence method.

[a]If the intra-subject variability in reference treatment <30% and the 90% CI of LS-mean ratio fell within 80.00% to 125.00%, PK bioequivalence was shown.

[b]If the intra-subject variability in reference treatment <30% and the 90% CI of LS-mean ratio fell within 80.00%-125.00%, PD equivalence was shown.

[c]Had to follow progesterone guidelines, which had to be modified to fit a partial replicated three-way crossover with six sequences.

FIGURE 3 Mean glucose infusion rate (GIR) profiles (per-protocol population for pharmacodynamics)



the trial, which was of mild severity, unrelated to study treatment, and resolved. There were no other clinically significant findings in physical examination, vital signs, ECGs, and haematology, biochemistry or urinalysis of the clinical laboratory tests assessed throughout the study.

The outcome of all TEAEs was resolved except one with the outcome resolving (mild infusion site thrombosis unrelated to Biocon's Insulin-N). No serious AEs or deaths occurred during the study.

4 | DISCUSSION

This study showed PK/PD equivalence of Biocon's Insulin-N and Humulin-N for the primary endpoints. Results of the secondary analyses also showed similarity between both drugs. A dose of 0.4 IU/kg body weight was considered a sensitive dose for intermediate-acting human insulin,[15] providing a strong dose-response relationship, and therefore was used in the current study.

This study was conducted in accordance with the EMA and FDA guidelines.[15,23] Healthy male and post-menopausal female subjects, who are representative of the most sensitive and appropriate trial population exhibiting lower intra-individual variability, were included.[15,23] Study subjects were per the inclusion and exclusion criteria specified in the protocol. However, the availability of healthy subjects at the study site, not excluding any race or gender per se, was the predominant factor determining the actual enrolment.

Applicable regulatory guidelines recommend a partially replicated three-period crossover design with replication of the reference product if high variability is anticipated.[15,22] However, this three-sequence design does not allow the estimation of the ICV of the test product, which is not replicated. Using a partially replicated three-period design with six sequences allows for replication of the test product, hence allowing estimation of both the test and the reference products' ICV. Although a fully replicated four-period crossover would have been the best design, collecting blood samples to characterize optimally the PK and PD profiles of NPH insulin during a 24-h clamp and over four trial periods raised ethical concerns because of the high blood volume required from each subject. The selection of a partially replicated three-period six-sequence design allowed a more accurate and robust estimation of test and reference products' ICV compared with a two-way (non-replicated) crossover design while minimizing the ethical concern of high blood volume loss associated with a fully replicated four-period design. The RSABE method allows to scale the bioequivalence window based on the subject variability of the reference drug. When using this approach, regulatory guidelines[22,24] require that subjects receive the reference drug more than once, e.g. in a replicated three period. The balance between this approach and an ethically acceptable number of subjects for this bioequivalence study was considered. The euglycaemic clamp setting for this study was based on an automated glucose clamp technique with continuous BG measurements and minute-by-minute adaptations of GIRs. This achieved the highest clamp quality possible while also reducing potential investigator-related bias and minimizing the risk of any drug-induced hypoglycaemia.[25] A clamp duration of 24 h was chosen to assess the complete PK and PD profiles of a single dose of both drugs.

The presence of endogenous insulin potentially interferes with the PK/PD assessments. Hence, certain measures were implemented along with the clamp technique to enable suppression of endogenous insulin: (a) using a higher than the recommended dose of 0.4 IU/kg for insulin doses in clamp studies, (b) suppressing endogenous insulin using a clamp target of 81 mg/dl ± 10%, which also avoided induction of hypoglycaemia/counter-regulatory hormones at the lowest end of the target range, (c) identifying subjects in whom endogenous insulin production potentially interfered with the PK/PD measurements by determining C-peptide levels in parallel with insulin concentrations, (d) using C-peptide-based correction methods for the primary analyses of PK parameters, and (e) using C-peptide-based exclusion rules for the primary analyses of PD parameters.

Both the Biocon Insulin-N and the Humulin-N were generally well tolerated, with no clinically relevant safety issues. Headache was the most commonly reported AE, which is a frequent AE in glucose clamp trials.[26–28] The asymptomatic hypoglycaemic events occurring after treatment administration resolved following an infusion of intravenous glucose. There were no noticeable differences in the safety profiles among the study drug formulations with regard to type, frequency and severity of AEs, local tolerability, vital signs, ECG and clinical laboratory results.

Diabetes management, a lifelong process, has a significant economic impact on health care systems.[29–31] In the United States, rising insulin prices prohibit access to affordable insulin, particularly for mid- to low-income individuals, those on high-deductible health plans or those who are uninsured.[11] According to a global survey involving 1478 respondents from 90 countries, insulin rationing is widespread.[32] Results showed that 18% (253 of 1408) of all respondents and nearly 26% (162 of 627) of the US respondents had rationed insulin at least once previously.[32]

The WHO steering group has observed that there is no significant difference in glycated haemoglobin, hypoglycaemic episodes, and other patient-related outcomes between analogues (glargine and detemir) and NPH in both T1D and T2D.[7,9,33,34] Many studies consider switching from insulin analogues to human insulins as these are safe and cost-effective in patients with T2D.[8,35] The introduction of Biocon's Insulin-N in this era of high cost of diabetes care can create affordable accessibility and hence adherence, thereby reducing the financial burden on the patient as well as the health care systems.

A predominantly male population was included in the study, which could be a possible limitation. As per the guideline's recommendations, the inclusion of only men in the studies is preferable, as insulin sensitivity in women may vary during the menstrual cycle. Further, majority of the study participants were European and White.

5 | CONCLUSION

This study showed equivalence between Biocon's Insulin-N and Humulin-N for the primary PK/PD endpoints when administered as a single subcutaneous injection. Results of the secondary PK/PD and safety endpoints showed that both insulin preparations were comparable, safe and well tolerated.

AUTHOR CONTRIBUTIONS

The study was designed by SNA, SL, AM and SMNM. Conduct/data collection was performed by GA, GS, NS and JP. GS, SL, AM and SMNM performed the analysis. All authors wrote and reviewed the manuscript, and all authors read and approved the final version of the manuscript.

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Biocon Biologics Ltd.). The author Sundara Moorthi Nainar Murugesan was employed with Biocon Biologics Ltd. at the time of study conduct and preparation of the manuscript. Sandeep N. Athalye, 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. The study was funded by Biocon Sdn BHd.

CONFLICT OF INTEREST

GA is an employee of Profil, Neuss, Germany. GS, AM, JP, SL and SNA are employees of Biocon Biologics Ltd and hold stocks in Biocon. NS is an employee of Biocon Biologics Ltd. SMNM is no longer employed by Biocon Biologics Ltd.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14994.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Grit Andersen https://orcid.org/0000-0001-9260-7165
Gursharan Singh https://orcid.org/0000-0003-2416-1266

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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