SYNOPSIS

Title of the study:	A randomized, cross-over, open, euglycemic clamp study on the relative bioavailability and activity of 0.6 U/kg insulin glargine and 20 µg lixisenatide, given as on-site mix compared to separate simultaneous injections in subjects with diabetes mellitus type 1 Study number: BDR11578
Investigator:	[redacted]
Study center:	1 center in Germany
Publications (reference):	N/A
Study period:	
Date first subject enrolled:	02-Jun-2010
Date last subject completed:	28-Jan-2011
Phase of development:	Exploratory (Phase 1)
Objectives:	
Primary:	To assess the relative bioavailability of a single dose of insulin glargine and lixisenatide given subcutaneously (SC) as an on-site mix versus separate and simultaneous injections of each drug.
Secondary:	To compare the activity of a single dose of insulin glargine and lixisenatide given SC as on-site mix versus given separately and simultaneously of each drug and to assess the safety and tolerability of insulin glargine and lixisenatide given SC as an on-site mix.
Methodology:	single-center, randomized, open-label, crossover (2 treatments, 2 treatment periods [TPs], and 2 sequences), euglycemic clamp study of the relative bioavailability and activity of single SC doses of 0.6 U/kg insulin glargine and 20 µg lixisenatide, administered as an on-site mix formulation, compared to their separate simultaneous injections, in subjects with type 1 diabetes mellitus (T1DM).
Number of subjects:	Planned: 22
	Randomized: 23
	Treated: 23
Evaluated:	Pharmacodynamic: 22
	Safety: 23
	Pharmacokinetics: 22
Diagnosis and criteria for inclusion:	Male and female subjects, between 18 and 65 years of age, inclusive, with T1DM for more than 1 year and total insulin dose of <1.2 U/kg per day.
Investigational product (T [Test]):	Solution for injection prepared on-site by mixing a fixed volume of the insulin glargine / lixisenatide premix solution for injection (800 µg/mL lixisenatide in 100 U/mL insulin glargine [Lantus U100]) with a variable volume of Lantus® U100, depending on subject's body weight.
Dose:	20 µg lixisenatide and 0.6 U/kg insulin glargine (Lantus), administered as 25 µl of the premix solution plus body-weight-adjusted volume of Lantus U100.
Administration:	SC, periumbilical; in fasted condition
Batch number(s):	[redacted]

Duration of treatment: Two days: single administrations of either T (a single injection of the on-site mix of lixisenatide and insulin glargine) or R (separate lixisenatide and insulin glargine injections) on Days 1 of TP1 and TP2, followed by a 24-hour euglycemic clamp.

Duration of observation: About 1 month (up to 7 months, including screening) for 1 subject: screening on Day (D)-28 to D-3; TP1 of 2 days (1 overnight stay); washout period of 5 to 18 days (preferably 7 days); TP2 of 2 days (1 overnight stay); and end-of-study (EOS) visit between D5 and D9 of TP2. In addition, a poststudy visit (PSV) at 4 to 6 weeks after last dosing and a follow-up visit (FUV) at 3 to 6 months (+2 weeks, per Amendment 1) following the PSV were conducted for anti-lixisenatide antibodies.

Reference therapy (R [Reference]): Solution for injection containing 100 U/mL insulin glargine (Lantus® U100).
Solution for injection containing 100 µg/mL lixisenatide.

Dose: 20 µg lixisenatide, 0.6 U/kg insulin glargine (Lantus®)

Administration: 2 separate SC (at opposite periumbilical sites), simultaneous (within 1 minute) injections; in fasted condition

Batch number(s): [redacted]

Criteria for evaluation:

Pharmacodynamic: The area under the body-weight-standardized glucose infusion rate (GIR) from time zero to 24 hours after dosing (GIR-AUC_{0-24}), the time to 50% of GIR-AUC_{0-24} ($t_{50\%}$-GIR-AUC_{0-24}), and also the maximum GIR (GIR_{max}) and the time to GIR_{max} (GIR-t_{max}).

Safety: Adverse events (AEs) reported by the subject or noted by the Investigator, vital signs, physical examination, standard hematology and blood chemistry parameters, urinalysis, electrocardiogram (12-lead and telemetry), local tolerability at the SC injection site, and anti-lixisenatide antibodies (at screening, TP1, TP2, and PSV and, in case of positive result, also at the FUV). Per Amendment 1, blood samples for anti-lixisenatide antibodies were collected for all subjects during the FUV, 3 to 6 months (+2 weeks) after the PSV, however, analyzed only if the respective PSV samples tested positive for the antibodies. An Allergic Reaction Assessment Committee was set up to adjudicate all allergic or possible allergic events. Subjects were closely monitored for any suspected acute pancreatitis (symptoms and signs of acute abdominal distress; serum amylase and lipase levels), which was to be documented as an AE. Adverse events with prespecified monitoring with immediate notification (QTc ≥500 ms, pregnancy, symptomatic overdose with investigational product (IP), pancreatitis and/or increase of pancreatic enzymes [amylase, lipase] >2 x ULN and without immediate notification (asymptomatic overdose with IP, local tolerability, allergic or allergic-like reaction) were collected.

Pharmacokinetic: Plasma concentrations of lixisenatide were used to determine the following pharmacokinetic (PK) parameters: maximum plasma concentration observed (C_{max}); first time to reach C_{max} (t_{max}); area under the plasma concentration versus time curve calculated using the trapezoidal method from time zero to the real time corresponding to the last concentration above the limit of quantification (AUC_{last}); area under the plasma concentration versus time curve extrapolated to infinity (AUC); apparent total body clearance (CL/F); apparent volume of distribution during the terminal phase (V_z/F); and terminal half-life associated with the terminal slope ($t_{1/2z}$).

Serum concentrations of insulin glargine were used to determine the following PK parameters: maximum serum concentration observed (C_{max}); t_{max} ; area under the serum concentration versus time curve calculated using the trapezoidal method from time zero to 24 hours after dosing (AUC_{0-24}); and the time to 50% of AUC_{0-24} ($t_{50\%}$- AUC_{0-24}).

Pharmacokinetic sampling times and bioanalytical methods: Blood was collected for the determination of plasma lixisenatide concentrations at time points 0H and 0H15, 0H30, 1H, 1H30, 2H, 2H30, 3H, 4H, 5H, 6H, 8H, 12H, and 24H after injection of study medication in both TPs. Double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) with lower limit of quantification (LLOQ) of 12 pg/ml was used.

Blood was collected for the determination of serum insulin glargine concentrations at time points 0H and 0H15, 0H30, 1H, 1H30, 2H, 4H, 6H, 8H, 10H, 12H, 14H, 16H, 18H, 20H, 22H, and 24H after injection of study medication in both TPs. Radioimmunoassay with LLOQ of 5.02 µU/mL (0.18 ng/mL or 30 pmol/L) was used.

Statistical methods:

Pharmacodynamics: Pharmacodynamic parameters were summarized by treatment using descriptive statistics. Statistical analysis compared treatments T and R. For GIR-AUC_{0-24}, the ratios between T and R treatments were assessed using a linear effects model for log-transformed values. Estimate and 90% confidence interval (CI) for the ratio of geometric means between treatments T and R were provided for GIR-AUC_{0-24}. Times to 50% of GIR-AUC_{0-24} ($T_{50\%}$-GIR-AUC_{0-24}) were compared nonparametrically between treatments T and R. GIR_{max} and GIR-t_{max} were subject to corresponding analyses albeit supplemental parameters. The analyses were conducted on the PD population (all subjects without any major deviations related to study drug administration, and for whom PD parameters were available).

Safety: The safety analysis was based on the review of individual values (clinically significant abnormalities) and descriptive statistics by treatment. For AEs, frequencies of subjects with treatment-emergent adverse events (TEAEs), coded according to the Medical Dictionary for Regulatory Activities (MedDRA) version 13.1 and classified by system-organ classes and preferred term, were tabulated by treatment. All AEs were listed. Statistical analysis of laboratory parameters, vital signs, and ECG used the potentially clinically significant abnormalities (PCSAs) criteria (Version 2.0, 14 September 2009). For vital signs and ECG, frequencies of subjects with abnormalities and PCSAs were summarized by treatment. Frequencies for signs of local intolerance were analyzed by treatment. The analyses were conducted on the safety population (all subjects who were exposed to any study treatment, regardless of the amount of treatment administered).

Pharmacokinetics: Pharmacokinetic parameters were summarized by treatment using descriptive statistics. Statistical analyses compared treatments T (on-site mix) and R (separate injections). The relative bioavailability between treatments T and R was assessed using a linear effects model for log-transformed AUC, AUC_{last}, and C_{max} values of lixisenatide and AUC_{0-24} values of insulin glargine. Estimate and 90% CI for the ratio of geometric means between treatments T and R was provided for these parameters. Time to 50% of AUC_{0-24} ($t_{50\%}$-AUC_{0-24}) was compared nonparametrically between treatments T and R. The analyses were conducted on the PK population (all subjects without any major deviations related to study drug administration, and for whom PK parameters were available).

Summary

- Pharmacodynamic results:
  Descriptive statistics for GIR-AUC_{0-24} and GIR_{max} and their treatment ratios are provided in the following 3 tables.
  GIR-AUC_{0-24} and GIR_{max} were numerically comparable between treatments, acknowledging that the lower boundary of the GIR-AUC_{0-24} treatment ratio CI slightly exceeded the 80% – 125% acceptance range.

Area under the body-weight-standardized glucose infusion rate time curve [GIR-AUC_{0-24} (mg/kg)] - descriptive statistics

	R (separate)	T (on-site mix)
GIR-AUC_{0-24h} (mg/kg)		
Number	22	21
Geometric Mean	1430.53	1277.44
CV%	54.063	84.018
Mean (SD)	1716.45 (927.96)	1862.52 (1564.86)
Median	1658.60	1467.10
Min : Max	308.1 : 4185.0	78.3 : 6464.8

R and T: insulin glargine 0.6 U/kg body weight and Lixisenatide 20 µg. T (test treatment) denotes injection of on-site mix of Lixisenatide and insulin glargine (Lantus®). R (reference treatment) denotes separate simultaneous injections of Lixisenatide and Lantus®.
During the calculation of the geometric mean all values equal to 0 have been defined as 1.

Maximum smoothed body-weight-standardized glucose infusion rate [GIR_{max} (mg/kg/min)] - descriptive statistics

	R (separate)	T (on-site mix)
GIR_{max} (mg/kg/min)		
Number	22	21
Geometric Mean	3.26	3.03
CV%	34.203	44.075
Mean (SD)	3.49 (1.20)	3.32 (1.46)
Median	3.49	2.79
Min : Max	1.0 : 5.5	1.4 : 6.8

GIR = body weight standardized glucose infusion rate / GIRmax and GIR-tmax are based on smoothed GIR profiles.

R and T: insulin glargine 0.6 U/kg body weight and Lixisenatide 20 µg. T (test treatment) denotes injection of on-site mix of Lixisenatide and insulin glargine (Lantus®). R (reference treatment) denotes separate simultaneous injections of Lixisenatide and Lantus®.

GIR-AUC and GIR_{max} estimates of treatment ratio with 90% confidence interval

Parameter	Comparison	Estimate	90% CI
GIR-AUC_{0-24h} [mg/kg]	T (on-site mix) / R (separate)	0.91	(0.71 to 1.17)
GIR_{max} [mg/kg/min]	T (on-site mix) / R (separate)	0.93	(0.82 to 1.06)

R and T: insulin glargine 0.6 U/kg body weight and lixisenatide 20 µg.

R (reference treatment) denotes separate simultaneous injections of lixisenatide and Lantus®.

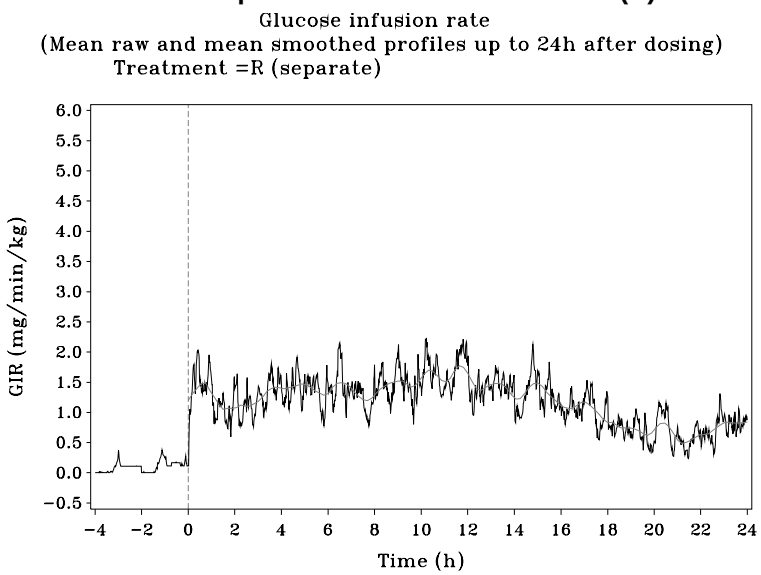
T (test treatment) denotes injection of on-site mix of lixisenatide and insulin glargine (Lantus®).

GIRmax is based on smoothed GIR profiles.

- GIR profiles

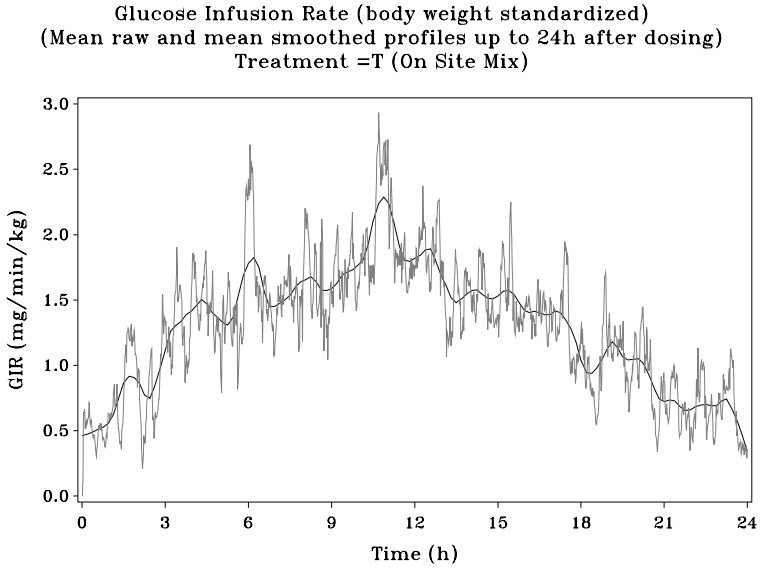
  Mean and median raw and smoothed GIR profiles for test treatment T and reference treatment R are presented in the figures below. The 24-hour GIR profiles are superimposable apart from the first hour of the postdose clamp period. The GIR for the reference treatment R, increased more rapidly after dosing at T0H as compared to the test treatment T. The conditions at the start of the on-treatment clamp at T0H were not different in both groups as indicated by the course of the pre-clamp profiles.

Mean GIR profiles – reference treatment (R)



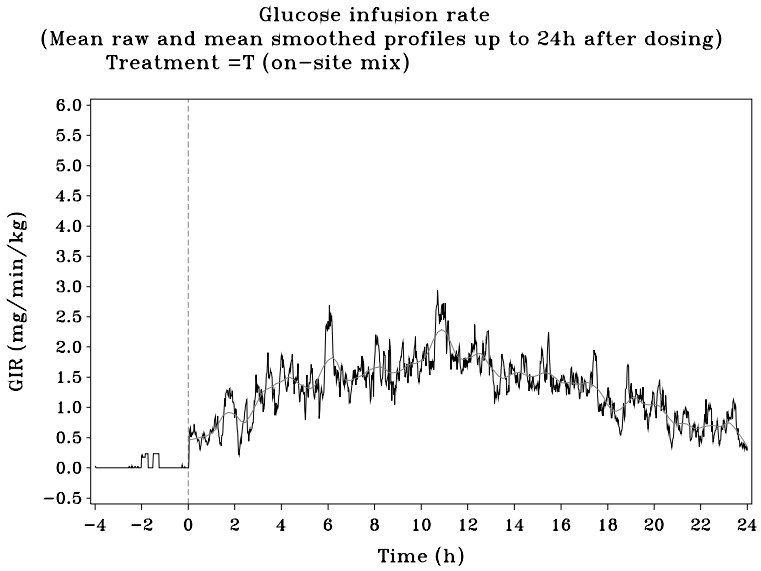
GIR = body weight standardized Glucose Infusion Rate.
R and T: insulin glargine 0.6 U/kg body weight and lixisenatide 20 µg. T (test treatment) denotes injection of on-site mix of lixisenatide and insulin glargine (Lantus®). R (reference treatment) denotes separate simultaneous injections of lixisenatide and Lantus®.

Mean GIR profiles – test treatment (T)



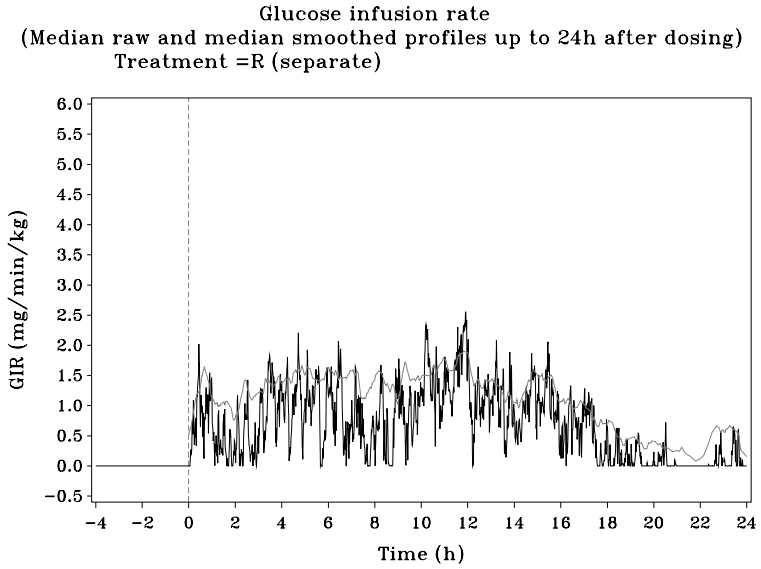
GIR = body weight standardized Glucose Infusion Rate
R and T: insulin glargine 0.6 U/kg body weight and Lixisenatide 20 µg. T (test treatment) denotes injection of on-site mix of Lixisenatide and insulin glargine (Lantus®). R (reference treatment) denotes separate simultaneous injections of Lixisenatide and Lantus®.

Mean GIR profiles – test treatment (T)



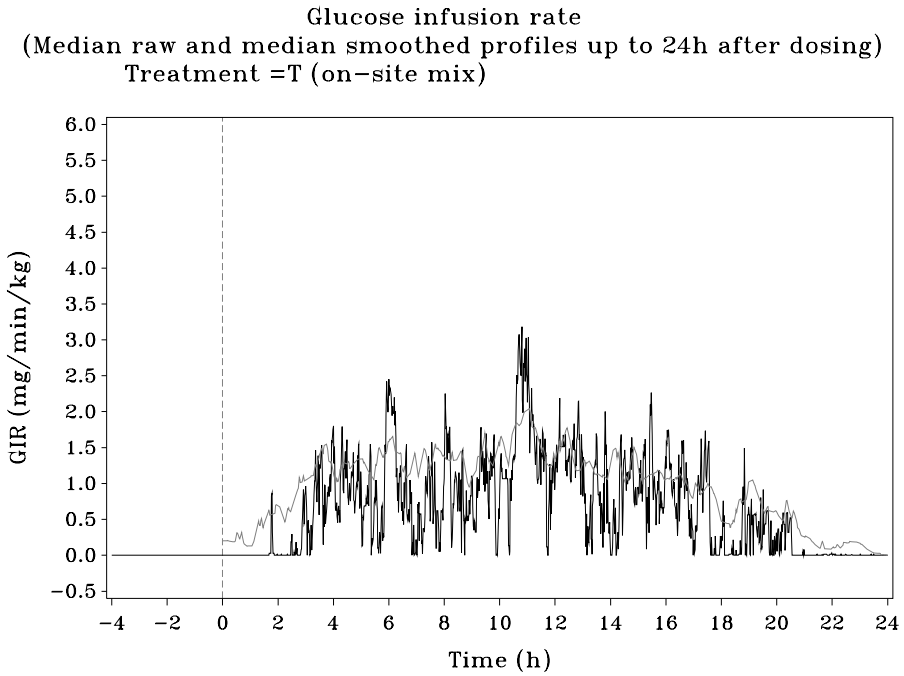
GIR = body weight standardized Glucose Infusion Rate.
R and T: insulin glargine 0.6 U/kg body weight and lixisenatide 20 µg. T (test treatment) denotes injection of on-site mix of lixisenatide and insulin glargine (Lantus®). R (reference treatment) denotes separate simultaneous injections of lixisenatide and Lantus®.

Median GIR profiles – reference treatment (R)



GIR = body weight standardized Glucose Infusion Rate.
R and T: insulin glargine 0.6 U/kg body weight and lixisenatide 20 µg. T (test treatment) denotes injection of on-site mix of lixisenatide and insulin glargine (Lantus®). R (reference treatment) denotes separate simultaneous injections of lixisenatide and Lantus®.

Median GIR profiles – test treatment (T)



GIR = body weight standardized Glucose Infusion Rate.
R and T: insulin glargine 0.6 U/kg body weight and lixisenatide 20 µg. T (test treatment) denotes injection of on-site mix of lixisenatide and insulin glargine (Lantus®). R (reference treatment) denotes separate simultaneous injections of lixisenatide and Lantus®.

Safety results: There were no SAEs, severe AEs, or TEAEs leading to treatment discontinuation. Seven and 9 subjects were reported to have a TEAE under T and R treatment, respectively. The most common TEAEs were headache, vomiting, and nausea. TEAEs of nausea and vomiting were equally distributed between both treatments. Potentially clinically significant abnormalities occurred infrequently with no trend observed in either treatment.

Under treatments R and T, 3 subjects each reported erythema that was barely perceptible. No associated irritation or pain was reported.

There were no positive anti-lixisenatide antibody samples.

- Pharmacokinetic results: Lixisenatide

Descriptive statistics for lixisenatide main pharmacokinetic parameters are provided in the first table below; corresponding treatment ratios and CIs are provided in the second table below.

The exposure to lixisenatide, AUC and AUC_{last} when given in combination with insulin glargine as an on-site mix of unbuffered lixisenatide in Lantus solution (T), met bioequivalence criteria compared to the reference treatment with separate injections (R). The maximum concentration, C_{max}, of lixisenatide given in combination as an onsite mix (T), did not meet formal bioequivalence criteria as the lower limit of the CI was outside the conventional acceptance range for bioequivalence.

Lixisenatide PK parameters - descriptive statistics

Mean ± SD (CV%) [geometric mean]	Plasma lixisenatide	
	R (separate)	T (on-site mix)
N	22	21
C_{max}	116 ± 24.7	100 ± 27.0
[pg/ml]	(21.3) [113]	(27.0) [96.2]
t_{max}[a]	2.00	2.50
[h]	(1.00 - 3.00)	(1.50 - 5.00)
AUC_{last}	531 ± 117	497 ± 126
[pg•h / ml]	(22.1) [519]	(25.2) [479]
AUC	601 ± 134	581 ± 134
[pg•h / ml]	(22.2) [587]	(23.1) [562][b]

a Median (Min - Max)
b n=20

Estimates of the treatment ratios (T/R) with 90% confidence intervals - lixisenatide

Parameter	Comparison	Estimate	90% CI
Cmax	T (on-site mix) vs. R (separate)	0.84	(0.74 to 0.96)
AUC	T (on-site mix) vs. R (separate)	0.96	(0.83 to 1.10)
AUClast	T (on-site mix) vs. R (separate)	0.92	(0.80 to 1.06)

R and T: insulin glargine 0.6 U/kg body weight and lixisenatide 20 µg.
R (reference treatment) denotes separate simultaneous injections of lixisenatide and Lantus®.
T (test treatment) denotes injection of on-site mix of lixisenatide and insulin glargine (Lantus®).

Insulin glargine
Descriptive statistics for insulin glargine main pharmacokinetic parameters are provided in Section 10.2.2, corresponding treatment ratio and CI for AUC is provided in the second table below and time to 50% AUC_{0-24h} is provided in the third table below. Exposure to insulin glargine was no different between the 2 treatments.

None of the insulin glargine PK parameters showed a relevant difference between the 2 treatments.

Insulin glargine PK parameters - descriptive statistics

Mean ± SD (CV%) [geometric mean]	Serum insulin_glargine	
	R (separate)	T (on-site mix)
N	22	21
C_{max}	16.6 ± 6.44	17.2 ± 6.72
[µU / ml]	(38.9) [15.5]	(39.1) [15.9]
t_{max}[a]	11.00	10.00
[h]	(8.00 - 14.00)	(2.00 - 18.00)
AUC_{0-24}	291 ± 99.4	302 ± 122
[µU•h / ml]	(34.1) [277]	(40.4) [279]

a Median (Min - Max)

Estimates of the treatment ratio (T/R) with 90% confidence interval – insulin glargine

Parameter	Comparison	Estimate	90% CI
AUC0-24	T (on-site mix) vs. R (separate)	1.01	(0.90 to 1.14)

R and T: insulin glargine 0.6 U/kg body weight and lixisenatide 20 µg.
R (reference treatment) denotes separate simultaneous injections of lixisenatide and Lantus®.
T (test treatment) denotes injection of on-site mix of lixisenatide and insulin glargine (Lantus®).

Insulin glargine - time to 50% AUC(0-24h) [T50%-AUC(0-24h) [h]] - descriptive statistics

	R	T
T50%-AUC(0-24h) (h)		
Number	22	21
Mean (SD)	11.918 (0.639)	11.770 (0.848)
Median	11.870	12.000
Min : Max	10.94 : 13.02	9.47 : 13.09

AUC = Area under the insulin glargine concentration time curve
R=(Reference) separate, T=(Test) on-site mix.

Conclusions: [redacted]

Date of report: 05-Oct-2015