SUPPLEMENTARY DATA

METHODS

Sampling

To prevent metabolic processing in sampled blood, venous blood was drawn into K_2-EDTA vials, immediately put on ice, and plasma obtained by centrifugation within the following 30 min and stored at - 20 °C.

Assay procedure

An aliquot (300 µL) of each plasma specimen was mixed with 50 µL of internal standard working solution [50 $\mu g.L^{-1}$ of [U-15N]-HOE901, 25 $\mu g.L^{-1}$ [U-15N]-HOE901-M1, $\mu g.L^{-1}$ [U-15N]-HOE901-M2 in acetonitrile/water/formic acid/Brij 35 (20:80:0.1:0.001, v/v/v/w)], 30 µL of acetonitrile/water/formic acid/Brij 35 (20:80:0.1:0.001, v/v/v/w) and 300 µL of 0.01 $mmol.L^{-1}$ PBS/Brij 35 (100:0.001, v/w) pH 7.8. The complete sample was loaded onto preconditioned immunoaffinity columns. After several washing steps HOE901, HOE901-M1 and HOE901-M2 was eluted with 300 µL of water/acetonitrile/formic acid/Brij 35 (90:10:1:0.001, v/v/v/w) and centrifuged through Vivacon 500 filters VN01 H02.

A total of 60 µL of the extract (300 µL in total) was injected into the liquid chromatography-tandem mass spectrometry (LC-MS/MS) system. Lower limit of quantification was 200 $\mu g.L^{-1}$ for insulin glargine, M1 and M2 corresponding to approximately 33 $pmol.L^{-1}$ or 5 $\mu U.mL^{-1}$. The LC-MS/MS system consisted of an API 5000 triple quadrupole mass spectrometer (AB Sciex, Darmstadt, Germany) equipped with a Turbo-V-source operating in positive mode and connected to an Ultimate 3000 HPLC system (Dionex, Idstein, Germany). An inlet valve was used to truncate non-relevant signals (10-port, VICI Valco Instruments, Houston, TX, USA). For the chromatography of insulin glargine, M1 and M2, a reversed phase column was used at 40 °C. A linear gradient was employed at a flow rate of 0.6 ml/min using water/formic acid (100:0.5, v/v) as mobile phase A and acetonitrile/formic acid (100:0.5, v/v) as mobile phase B. Total run time was 8.25 min. Retention time of insulin glargine, M1 and M2 and that of the matching internal standards $^{15}N_{72}$-insulin glargine, $^{15}N_{64}$-M1 and $^{15}N_{63}$-M2 were 2.07, 2.13 and 2.13 min, respectively. The mass spectrometer was operated in the positive ion mode with an electrospray voltage of +5 500 V at 600 °C. Nebulizer gas (GS1) was set to 80 psi, heater gas (GS2) to 90 psi and curtain gas to 50 psi. Collision gas was set to 7 instrument units. Position of the electrospray needle was 5 mm (horizontal) and 5 mm (vertical). Multiple reactions monitoring (MRM) was used for quantification. The mass transitions used were m/z 867.0 → m/z 136.1 for insulin glargine, m/z 959.7 → m/z 136.1 for M1, m/z 942.9 → m/z 136.1 for M2, m/z 877.4 → m/z 137.2 for $^{15}N_{72}$-insulin glargine, m/z 970.0 → m/z 137.2 for $^{15}N_{64}$-M1 and m/z 953.0 → m/z 137.2 for $^{15}N_{63}$-M2. All quadrupoles were working at unit resolution. Quantitation was performed with Analyst Software V1.4.2 (AB Sciex, Darmstadt, Germany) using the internal standard method. Ratios of analyte peak area and internal standard peak area (y-axis) were plotted against concentration (x-axis), and calibration curves were calculated by least square regression with $(concentration)^{-2}$ weighting. There was no cross reactivity of the LC-MS/MS assay against human insulin, insulins lispro, glulisine, aspart or detemir. Accuracy and precision were assessed on six separate occasions at 0.2, 0.6, 5 and 8 $mg.L^{-1}$ for glargine, M1 and M2. The total precision ranged from 4.3% to 11.2% for glargine, from 4.7% to 11.1% for M1 and from 3.6% to 8.4% for M2.

SUPPLEMENTARY DATA

Supplementary Table 1. Clinical characteristics of subjects with T1DM (mean ± SD)				
Glargine Dose (U.kg^{-1})	0.3	0.6	1.2	All
N	12	11	11	34
Age (y)	38.6 (10.4)	40.0 (11.7)	37.1 (10.0)	38.6 (10.5)
Weight (kg)	84.9 (11.3)	82.1 (10.7)	82.3 (10.2)	83.2 (10.5)
BMI (kg.m^{-2})	25.7 (2.3)	25.8 (2.3)	24.8 (2.2)	25.4 (2.3)
HbA1c (mmol.mol^{-1})	64 (17)	60 (16)	60 (14)	61 (16)
HbA1c %	*8.0 (0.6)*	*7.6 (0.7)*	*7.6 (0.9)*	*7.7 (0.7)*
IFCC-HbA1c (mmol.mol^{-1}) = [DCCT-HbA1c (%) - 2.15] x 10.929 mmol.mol^{-1}				

Supplementary Figure 1. *Individual plasma concentrations of insulin glargine (M0, left panel), Des-30^{B}-Thre-21^{A}-Gly human insulin (M2, middle panel) and 21^{A}-Gly human insulin (M1, right panel) after s.c. injection of 0.3, 0.6, and 1.2 U.kg^{-1} insulin glargine in subjects with T1DM. Symbol lines correspond to subjects with detectable M0 and/or M2 specified by number, dotted lines to all with M1 only, and dashed lines to LLOQ (33 pmol.L^{-1}).*