

Supplemental data:

Chemicals and materials

Acetonitrile (ACN, art.nr. 1207801) and formic acid (art.nr 6914143) were purchased from Biosolve (Valkenswaard, The Netherlands). Leucine enkephaline (art.nr L9133) and octyl- β -glucoside (art.nr. 850511P) were purchased from Sigma Aldrich (Zwijndrecht, The Netherlands). MSIA D.A.R.T (art nr 991001096) and tri-fluoroacetic acid (TFA) were purchased from Thermo Scientific (Waltham, MA, USA). Phosphate buffered saline (PBS, art.nr 13763806) was purchased from Fresenius Kabi (Huis ter Heide, The Netherlands). The internal standard (IS) 4-[D10-leu] insulin (art.nr PLP-3404-V) was purchased from Peptides International (Louisville, KY, USA). Insulin glulisine (Apidra), and insulin glargine (Lantus) were purchased from Sanofi-Aventis (Paris, France). Insulin detemir (Levemir), insulin aspart (Novorapid), human insulin (Actrapid) and insulin tresiba (Degludec) were purchased from Novo-Nordisk (Bagsværd, Denmark). Insulin Lispro (Humalog) was purchased from Eli Lilly (Indianapolis, IN, USA). All insulin analogs were purchased at a concentration of 100 IU/mL. Lantus Metabolite 1 was kindly provided by Sanofi-Aventis (Frankfurt am Main, Germany). Calibrators for the Atellica insulin assay (art.nr 1099562) were purchased from Siemens (Siemens-Healthineers, Den Haag, The Netherlands). UPLC grade water was delivered by a Pure Flex system from Elga (Veolia Water Solutions & Technologies, Ede, The Netherlands).

S1: MSIA D.A.R.T. protocol

Step	Volume (uL)	Speed	Cycles
Wash (PBS)	150	4	20
Capture	250	1	500
Wash (PBS)	150	4	20
Wash (PBS)	150	4	20
Wash (H ₂ O)	150	4	20
Wash (H ₂ O)	150	4	20
Elute (33% ACN, 0.4% TFA, 190 ug/mL Leucine Enkephaline)	50	1	100

S2: LC instrument parameters

Instrument	Waters Acquity UPLC system		
Pump	Binary Solvent Manager		
Column	Waters Cortecs UPLC C18+		
Column temperature (°C)	60		
Column dimensions	50 x 2.1 mm, 1.7 µm		
Mobile Phase A	H ₂ O + 0.1% formic acid		
Mobile Phase B	Acetonitrile + 0.1% formic acid		
Flow (mL/min)	0.250		
Gradient	Time (min)	%B	Curve
	Initial	15	Initial
	2.00	15	6
	5.00	40	6
	5.10	90	6
	6.10	90	6
	6.50	15	6
	8.50	15	6
Injector	Fixed Loop		
Injection volume (µL)	25		

S3: Waters Xevo-TQ-S MS instrument parameters

Source	Ion mode	ESI+
	Capillary voltage	2.5 kV
	Source offset	50 V
	Source temperature	150 °C
	Desolvation temperature	600 °C
	Desolvation gas flow (N ₂)	1000 L/hr
	Cone gas flow (N ₂)	150 L/hr
Analyzer 1	LM resolution	2.5
	HM resolution	13.5
	Ion energy 1	0.2
Collision cell	Collision gas (Ar)	0.15 mL/min
	Entrance	1
	Collision energy	Variable
	Exit	1
Analyzer 1	LM resolution	2.5
	HM resolution	13.5
	Ion energy 2	0.8

S4: MRM parameters

Component	MRM	Cone voltage (V)	Collision energy (eV)
Endogenous insulin			
Quantifier	968.7>226.2	35	45
Qualifier	1162.0>226.2	35	45
4-[D10-Leu] insulin (IS)			
Quantifier	975.0>226.0	40	30
Qualifier	1170.0>226.0	60	30
Apidra			
Quantifier	1165.4>1370.0	40	22
Qualifier	1165.4>346.2	14	22
Degludec			
Quantifier	1221.6>641.4	20	25
Qualifier	1018.2>1093.2	20	15
Detemir			
Quantifier	1184.2>454.5	40	28
Qualifier	1184.1>1366.7	40	22
Humalog			
Quantifier	1162.3>217.2	50	40
Qualifier	968.2>217.0	50	40
Lantus Metabolite 1			
Quantifier	959.4>1128.4	20	20
Qualifier	959.4>1118.4	20	21
Novorapid			
Quantifier	971.8>660.9	50	18
Qualifier	971.8>1139.4	12	18

S5: Analytical performance characteristics

	Novorapid	Apidra	Humalog	Detemir	Degludec	Lantus M1
Accuracy (%)	95	97	102	109	74	103
Precision (%)						
Concentration (range 15-1200 pmol/L)	12	8	11	25	21	18
1 st target ratio	6	4	8	6	27	5
Carry-over (%)	0.4	0.6	0.3	1.5	2.1	0.4

Supplemental Figure 1:

Passing and Bablok regression of the LC-MS/MS method versus the Atellica insulin assay (Siemens-Healthineers, Den Haag, The Netherlands) for the determination of endogenous insulin using left-over patient samples (N=10).

