Quantification of Insulin at Microdosing Levels Using LC-MS/MS

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Overview

Purpose: Development of a robust analytical method for the quantitation of human insulin.

Methods: An LC-MS/MS method employing the TSQ Vantage™ triple quadrupole mass spectrometer is used to detect bovine and human insulin with a 3 min gradient from 95 % water (Fisher Scientific HPLC grade) (0.1 % formic acid) to 100% acetonitrile (Fisher Scientific HPLC grade) (0.1 % formic acid) at a flow of 750 µL /min.

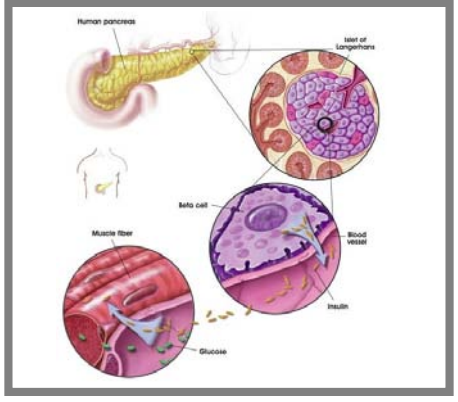
Results: A robust LC-MS/MS method with a calibration curve of 5 orders of magnitude dynamic range starting from 30 amol/mL is demonstrated using the TSQ Vantage.

Introduction

Insulin is a protein hormone, secreted by the beta (or B) cells of the islets of Langerhans in the pancreas, that promotes the uptake of glucose by body cells, particularly in the liver and muscles, and thereby controls its concentration in the blood.

Insulin is a tiny protein consisting of two linked polypeptide chains derived from a single proinsulin chain. The two chains, "A" consisting of 21 amino acids and "B" of 30 amino acids, are linked by two disulfide bridges. Insulin was the first protein to have its amino acid sequence determined (in 1955). Underproduction of insulin results in the accumulation of large amounts of glucose in the blood and its subsequent excretion in the urine. This condition, known as diabetes mellitus, can be treated successfully by insulin injection.[1]

FIGURE 1. Insulin production in the human pancreas.

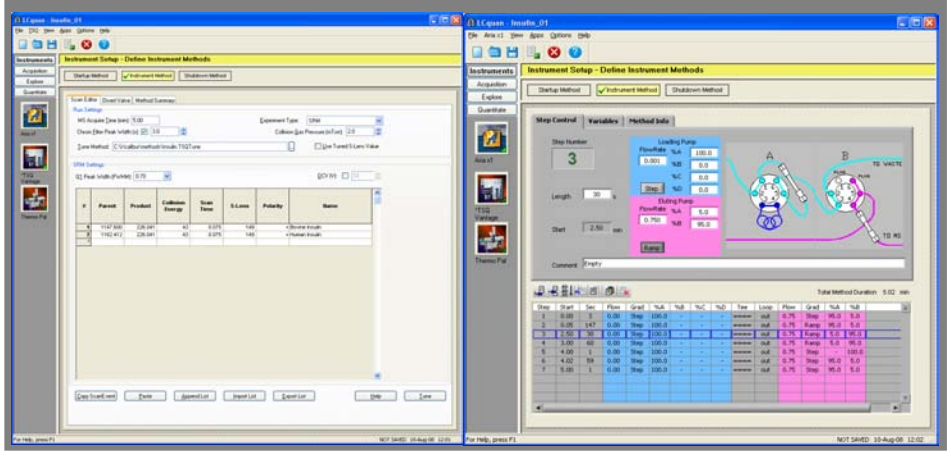


The number of people diagnosed with diabetes has increased tremendously the last decade, especially in countries such as India and China. Bio-equivalence studies of non-endogenous insulin in human clinical studies requires a robust, reproducible, highly specific and highly sensitive, LC-MS/MS method. Previous methods used capillary LC to increase sensitivity necessary to reach the required detection levels, however, these methods were tedious, not very robust, and variable. This poster describes a robust LC-MS/MS method using normal flow rates (300-800uL/min) and standard electrospray techniques achieving attomoles per milliliter levels of detection.

Methods

Human insulin (Sigma Aldrich MW 5,808) in a rat serum (Lampire Biological laboratories) matrix was analyzed. Bovine insulin (Sigma Aldrich, MW 5,777) was used as an internal standard. The column used was a Thermo Scientific BioBasic-4, 100 X 2.1 mm packed with 5 um particles. A fast 3 min gradient of 95 % water (0.1 % formic acid) to 100% acetonitrile (0.1 % formic acid) at a flow of 750 µL /min was used to elute both insulin from the column. The transitions used for measuring the insulin and its internal standard were m/z 1162.4→ 226.2, 315.2 and m/z 1147.5 → 226.2, 315.2 respectively. The collision energy necessary for optimal fragmentation was 43 volts. Spiked rat serum was used as a sample matrix to create the calibration curve and to determine the detection level. QCs at three levels (200 amol/µL 9 and 450 pmol/µL.) See Figure 2 for the TSQ Vantage (Thermo Fisher Scientific) mass spectrometer and Aria LC (Thermo Fisher Scientific) instrument methods

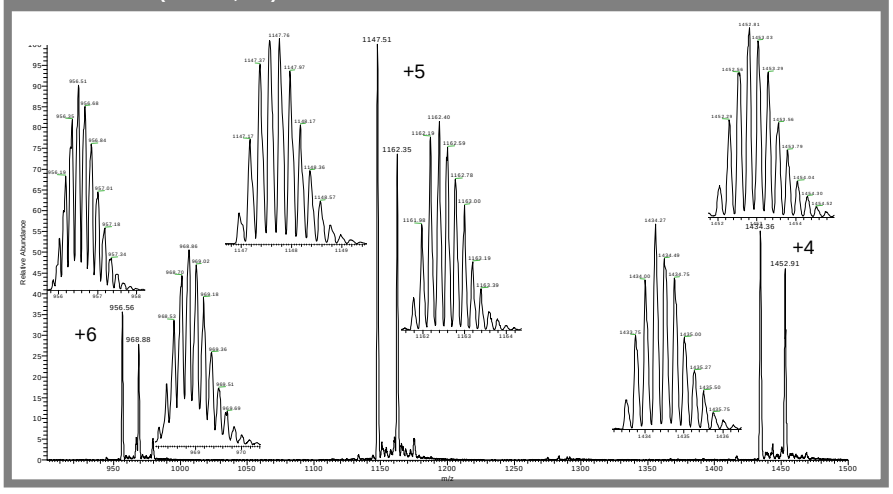
FIGURE 2. TSQ Vantage instrument Method (left) and the Aria LC Method (right)



Results

In Figure 3 a full scan spectrum of a mixture of both the insulin species as acquired with the TSQ Vantage. The insets with higher resolution are to determine the charge states. From this spectrum we decided that both 5^+ charge state species m/z 1162.4 for the Human insulin and m/z 1147.5 for the Bovine insulin were the most suitable peaks for the development of the experiments.

FIGURE 3. Full scan spectrum and charge state determination of human (MW = 5,808) and bovine insulin (MW = 5,777) .



A quadratic fitted calibration curve from 30 amol/µL to 3 pmol/µL was obtained by using crashed rat serum as a matrix. The transitions used for measuring the insulin and its internal standard were m/z 1162.2→ 226.2, 315.2 and m/z 1148 → 226.2, 315.2 respectively. The collision energy necessary for optimal fragmentation is 43 volts. The calibration curve has good linearity over 5 orders of magnitude. The LOQ of the method was 30 amol/mL. Reproducibility at the LLOQ of the curve is 9% RSD. The precision and reproducibility of the 130 injected QCs are well within the FDA guidelines.

FIGURE 4. Calibration curve of Human Insulin

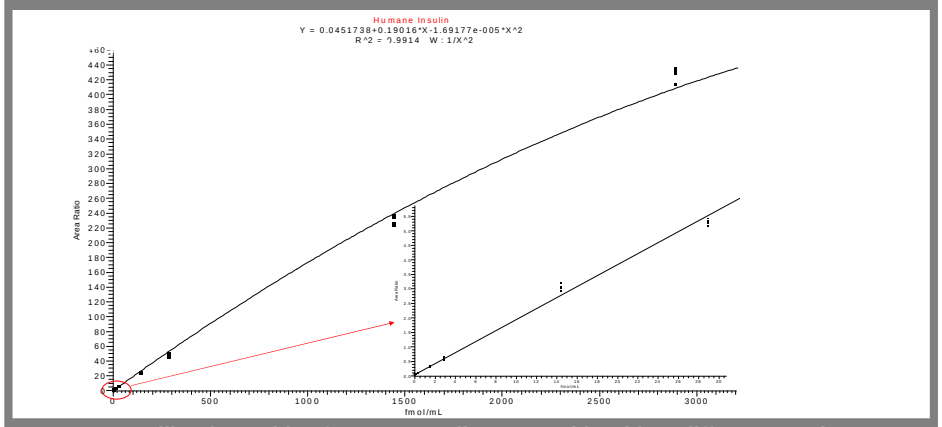


Table 4 & 5. Calibration Table of Human Insulin & QC Table with % difference and %RSDs

FileName	Area	Level	Spike Conc.	Calc. Conc	% Diff	% RSD
Insulin_0006	392	2	0.029	0.0262	-9.22	9.53
Insulin_0007	389	2	0.029	0.0319	10.41	9.53
Insulin_0008	403	2	0.029	0.0292	1.20	9.53
Insulin_0009	317	2	0.029	0.0252	-12.94	9.53
Insulin_0010	394	2	0.029	0.0294	1.88	9.53
Insulin_0011	432	3	0.145	0.1605	10.66	6.78
Insulin_0012	543	3	0.145	0.1570	8.29	6.78
Insulin_0013	510	3	0.145	0.1404	-3.15	6.78
Insulin_0014	494	3	0.145	0.1463	0.86	6.78
Insulin_0015	523	3	0.145	0.1376	-5.09	6.78
Insulin_0016	742	4	0.289	0.3289	13.79	2.74
Insulin_0017	738	4	0.289	0.3311	14.55	2.74
Insulin_0018	647	4	0.289	0.3278	13.42	2.74
Insulin_0019	650	4	0.289	0.3091	6.94	2.74
Insulin_0020	721	4	0.289	0.3221	11.46	2.74
Insulin_0021	2708	5	1.445	1.3680	-5.33	5.00
Insulin_0022	2808	5	1.445	1.4901	3.12	5.00
Insulin_0023	2713	5	1.445	1.4611	1.11	5.00
Insulin_0024	2510	5	1.445	1.5452	6.94	5.00
Insulin_0025	2832	5	1.445	1.5488	7.19	5.00
Insulin_0026	5372	6	2.890	3.1366	8.53	7.42
Insulin_0027	5022	6	2.890	2.9226	1.13	7.42
Insulin_0028	5000	6	2.890	2.7804	-3.79	7.42
Insulin_0029	4901	6	2.890	3.0597	5.87	7.42
Insulin_0030	4571	6	2.890	2.6019	-9.97	7.42
Insulin_0031	25884	7	14.450	15.9101	10.10	3.44
Insulin_0032	25186	7	14.450	15.8563	9.73	3.44
Insulin_0033	26335	7	14.450	16.6720	15.38	3.44
Insulin_0034	23857	7	14.450	15.6551	8.34	3.44
Insulin_0035	24029	7	14.450	15.1629	4.93	3.44
Insulin_0036	43768	8	28.900	26.9971	-6.58	2.03
Insulin_0037	49844	8	28.900	28.3049	-2.06	2.03
Insulin_0038	50164	8	28.900	27.0467	-6.41	2.03
Insulin_0039	40082	8	28.900	27.4876	-4.89	2.03
Insulin_0040	48395	8	28.900	27.8931	-3.48	2.03
Insulin_0041	240956	9	144.500	134.4000	-6.99	4.83
Insulin_0042	238344	9	144.500	130.1590	-9.92	4.83
Insulin_0043	189245	9	144.500	119.1433	-17.55	4.83
Insulin_0044	232620	9	144.500	134.4405	-6.96	4.83
Insulin_0045	195222	9	144.500	130.6687	-9.57	4.83
Insulin_0046	412128	10	289.000	263.4452	-8.84	5.28
Insulin_0047	392114	10	289.000	264.1641	-8.59	5.28
Insulin_0048	465465	10	289.000	272.7203	-5.63	5.28
Insulin_0049	394706	10	289.000	236.8490	-18.05	5.28
Insulin_0050	380137	10	289.000	254.4252	-11.96	5.28
Insulin_0051	1875746	11	1445.000	1398.0368	-3.25	3.11
Insulin_0052	1763302	11	1445.000	1347.5079	-6.75	3.11
Insulin_0053	1818564	11	1445.000	1329.8404	-7.97	3.11
Insulin_0054	2141546	11	1445.000	1333.6513	-7.71	3.11
Insulin_0055	2130953	11	1445.000	1425.4134	-1.36	3.11
Insulin_0056	3940626	12	2890.000	3119.8063	7.95	3.04
Insulin_0057	3794057	12	2890.000	2953.8957	2.21	3.04
Insulin_0058	3191827	12	2890.000	3203.0780	10.83	3.04
Insulin_0059	3816244	12	2890.000	3113.9820	7.75	3.04
Insulin_0060	3754989	12	2890.000	3161.6409	9.40	3.04

FileName	Level	% Diff	% RSD	FileName	Level	% Diff	% RSD
Insulin_100	QC Low	19.18	14.22	Insulin_166	QC Mid	-1.26	5.71
Insulin_101	QC Low	1.79	14.22	Insulin_167	QC Mid	-6.14	5.71
Insulin_102	QC Low	-7.98	14.22	Insulin_168	QC Mid	-9.45	5.71
Insulin_103	QC Low	4.79	14.22	Insulin_169	QC Mid	3.60	5.71
Insulin_104	QC Low	10.33	14.22	Insulin_170	QC Mid	-4.29	5.71
Insulin_105	QC Low	12.76	14.22	Insulin_171	QC Mid	-7.12	5.71
Insulin_106	QC Low	7.03	14.22	Insulin_172	QC Mid	5.19	5.71
Insulin_107	QC Low	13.81	14.22	Insulin_173	QC Mid	-3.90	5.71
Insulin_108	QC Low	4.27	14.22	Insulin_174	QC Mid	2.85	5.71
Insulin_109	QC Low	-2.62	14.22	Insulin_175	QC Mid	11.79	5.71
Insulin_110	QC Low	-18.97	14.22	Insulin_176	QC Mid	2.15	5.71
Insulin_111	QC Low	10.68	14.22	Insulin_177	QC Mid	2.38	5.71
Insulin_112	QC Low	-17.92	14.22	Insulin_178	QC Mid	-2.34	5.71
Insulin_113	QC Low	-18.35	14.22	Insulin_179	QC Mid	-3.80	5.71
Insulin_114	QC Low	-18.42	14.22	Insulin_180	QC Mid	-1.29	5.71
Insulin_115	QC Low	-7.70	14.22	Insulin_181	QC Mid	8.04	5.71
Insulin_116	QC Low	-9.46	14.22	Insulin_182	QC Mid	-0.93	5.71
Insulin_117	QC Low	19.39	14.22	Insulin_183	QC Mid	-1.50	5.71
Insulin_118	QC Low	-17.98	14.22	Insulin_184	QC High	-4.40	3.83
Insulin_119	QC Low	7.23	14.22	Insulin_185	QC High	-0.43	3.83
Insulin_120	QC Low	8.18	14.22	Insulin_186	QC High	3.12	3.83
Insulin_121	QC Low	14.83	14.22	Insulin_187	QC High	-4.62	3.83
Insulin_122	QC Low	-19.22	14.22	Insulin_188	QC High	5.33	3.83
Insulin_123	QC Low	-17.31	14.22	Insulin_189	QC High	-1.28	3.83
Insulin_124	QC Low	-13.76	14.22	Insulin_190	QC High	-1.81	3.83
Insulin_125	QC Low	-19.39	14.22	Insulin_191	QC High	-5.43	3.83
Insulin_126	QC Low	0.33	14.22	Insulin_192	QC High	-1.80	3.83
Insulin_127	QC Low	18.77	14.22	Insulin_193	QC High	-0.95	3.83
Insulin_128	QC Low	17.36	14.22	Insulin_194	QC High	2.02	3.83
Insulin_129	QC Low	-10.22	14.22	Insulin_195	QC High	-6.02	3.83
Insulin_130	QC Low	0.64	14.22	Insulin_196	QC High	0.15	3.83
Insulin_131	QC Low	-17.82	14.22	Insulin_197	QC High	-5.43	3.83
Insulin_132	QC Low	18.35	14.22	Insulin_198	QC High	-3.34	3.83
Insulin_133	QC Low	-17.33	14.22	Insulin_199	QC High	1.26	3.83
Insulin_134	QC Low	-18.46	14.22	Insulin_200	QC High	3.89	3.83
Insulin_135	QC Low	-1.73	14.22	Insulin_201	QC High	-1.02	3.83
Insulin_136	QC Mid	-3.01	5.71	Insulin_202	QC High	1.73	3.83
Insulin_137	QC Mid	-8.12	5.71	Insulin_203	QC High	1.33	3.83
Insulin_138	QC Mid	-4.19	5.71	Insulin_204	QC High	3.49	3.83
Insulin_139	QC Mid	4.40	5.71	Insulin_205	QC High	2.25	3.83
Insulin_140	QC Mid	-1.50	5.71	Insulin_206	QC High	1.86	3.83
Insulin_141	QC Mid	-2.00	5.71	Insulin_207	QC High	2.23	3.83
Insulin_142	QC Mid	4.44	5.71	Insulin_208	QC High	-1.92	3.83
Insulin_143	QC Mid	-4.79	5.71	Insulin_209	QC High	-5.23	3.83
Insulin_144	QC Mid	5.74	5.71	Insulin_210	QC High	3.30	3.83
Insulin_145	QC Mid	6.60	5.71	Insulin_211	QC High	1.17	3.83
Insulin_146	QC Mid	3.31	5.71	Insulin_212	QC High	-3.93	3.83
Insulin_147	QC Mid	-1.25	5.71	Insulin_213	QC High	1.76	3.83
Insulin_148	QC Mid	12.94	5.71	Insulin_214	QC High	-9.21	3.83
Insulin_149	QC Mid	0.17	5.71	Insulin_215	QC High	-1.93	3.83
Insulin_150	QC Mid	-4.37	5.71	Insulin_216	QC High	-0.39	3.83
Insulin_151	QC Mid	2.84	5.71	Insulin_217	QC High	-3.04	3.83
Insulin_152	QC Mid	3.17	5.71	Insulin_218	QC High	-10.99	3.83
Insulin_153	QC Mid	-7.28	5.71	Insulin_219	QC High	-3.23	3.83
Insulin_154	QC Mid	7.59	5.71	Insulin_220	QC High	-3.16	3.83
Insulin_155	QC Mid	6.85	5.71	Insulin_221	QC High	1.65	3.83
Insulin_156	QC Mid	-10.65	5.71	Insulin_222	QC High	-8.04	3.83
Insulin_157	QC Mid	10.08	5.71	Insulin_223	QC High	-4.51	3.83
Insulin_158	QC Mid	3.22	5.71	Insulin_224	QC High	1.38	3.83
Insulin_159	QC Mid	-6.07	5.71	Insulin_225	QC High	1.00	3.83
Insulin_160	QC Mid	1.30	5.71	Insulin_226	QC High	0.24	3.83
Insulin_161	QC Mid	-5.50	5.71	Insulin_227	QC High	1.95	3.83
Insulin_162	QC Mid	0.85	5.71	Insulin_228	QC High	3.06	3.83
Insulin_163	QC Mid	-1.72	5.71	Insulin_229	QC High	3.34	3.83
Insulin_164	QC Mid	-7.54	5.71	Insulin_230	QC High	0.02	3.83
Insulin_165	QC Mid	8.54	5.71	Insulin_231	QC High	6.72	3.83

Conclusions

A highly sensitive and robust method can be developed for biosimilars like Insulin on the new TSQ Vantage triple quadrupole with normal bioanalytical flow rates. The method on the TSQ Vantage has a large dynamic range of five orders of magnitude, with accuracies and reproducibilities well within the guidelines of the FDA.

References

1. The internet encyclopedia of science