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Development of bioanalytical method for determination of intact human insulin from plasma using LC/MS/MS

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Introduction

Human insulin is a peptide hormone composed of 51 amino acids, and has a molecular weight of 5808 Da. It is a heterodimer of two peptide chains connected by disulphide bonds that is secreted by beta cells of pancreas, and is central to regulating carbohydrate and fat metabolism in the body. Animal form insulin, including porcine and bovine insulin, has been used clinically for the treatment of diabetes. However, biosynthetic human insulin is preferred because side reactions are generally less common [1].

Quantitation of insulin from biological fluids is important to understand its pharmacokinetics. Traditionally, measurement of this peptide hormone is carried out using immunoassays such as ELISA or RIA. However, the major drawbacks of these techniques are cross reactivity, prolonged assay development times, reagent optimization, reagent procurement and matrix effects. LC/MS/MS technique which has been established as a gold standard for small molecule quantitation, is now emerging as a tool for quantitation of bio-therapeutics as well. In comparison to conventional LBAs', LC/MS/MS based approach offers many advantages in terms of selectivity by MRM, improved throughput by multiplexed detection of targets, wide dynamic range, short development time lowering cost of analysis etc.

However, few challenges still exist in quantitation of proteins using LC/MS/MS such as nonspecific binding, poor fragmentation etc. Different approaches of extraction, separation and detection of human insulin have been reported in the literature such as enrichment using immunocapture, SPE, quantitation of insulin B chain by reducing intact insulin, use of low-flow HPLC, 2D-LCMS etc [2]. Here, simple SPE technique was used for sample pretreatment and LC/MS/MS method was developed for quantitation of intact human insulin in plasma using LCMS-8060, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan.

Methods and Materials

Sample preparation

Intact human insulin and bovine insulin IS (Sigma-Aldrich) were spiked in charcoal stripped human plasma over a concentration range of 15 to 500 pM/L. Charcoal stripped plasma was used in order to remove endogenous insulin interference. LQC (15 pM/L) , MQC (50 pM/L) and HQC (300 pM/L) levels were also prepared in charcoal stripped human plasma. All the linearity insulin standards and QC samples were processed using HLB SPE cartridges.

LC/MS/MS analysis

Intact human insulin was analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera coupled with LCMS-8060 triple quadrupole system (Shimadzu Corporation, Japan) shown in Figure 1.

LCMS-8060 triple quadrupole mass spectrometer, sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

In order to improve ionization efficiency, the newly developed heated ESI probe (shown in Figure 2) combines high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and enhancing ionization. This development allows high-sensitivity analysis of a wide range of target compounds with considerable reduction in background.

Details of analytical conditions are given in Table 1.

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Figure 1. Nexera with LCMS-8060

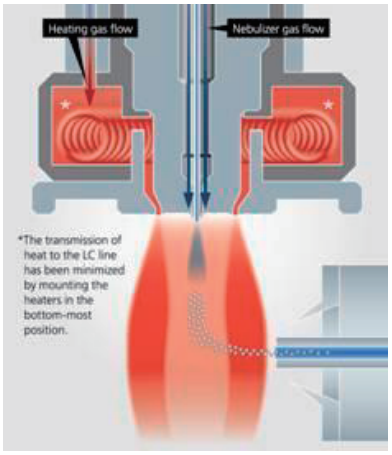

Figure 2. Heated ESI probe

Table 1. LC/MS/MS conditions for intact human insulin

Column	: C18 column
Mobile phase	: A: 0.1 % formic acid in water
	B: 0.1 % formic acid acetonitrile
Elution mode	: gradient
Oven temperature	: 40 °C
MS interface	: Electro Spray Ionization (ESI)
Nebulizing gas flow	: 3 L/min
Drying gas flow	: 10 L/min
Heating gas flow	: 18 L/min
Desolvation line temperature	: 200 °C
Heat block temperature	: 400 °C
Interface temperature	: 350 °C

Results

Intact human insulin was analysed on LCMS-8060 in ESI positive mode. For quantitative analysis of intact human insulin, the MH5 +5 ion of m/z 1162.30 was used as a precursor ion and m/z of 1158.90 was selected as product ion. Linearity study was carried out using internal standard calibration method. Intact bovine insulin was used as internal standard and MRM transition of 956.40 >136.30 was selected for internal standard peptide. MRM transitions were optimized using automatic MRM optimization feature of LabSolutions.

LOQ for intact human insulin was determined based on the following criteria – (1) % RSD for area ratio < 20 %, (2) % accuracy between 80-120 % and (3) Signal to noise ratio (S/N) > 10. Result of linearity study from 15 to 500 pM/L is shown in Figure 3. MRM chromatograms of linearity levels and QC samples prepared in charcoal stripped human plasma are shown in Figures 4 and 5 respectively. No peak was seen in blank charcoal stripped human plasma at the retention time of intact human insulin, which confirms the absence of any interference. Precision and accuracy results are tabulated in Table 2.

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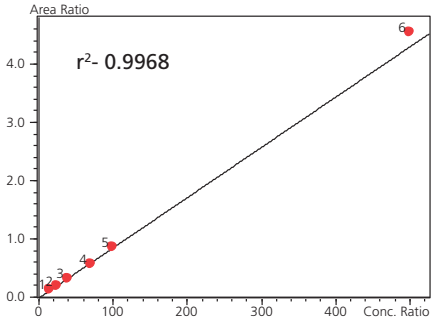

Figure 3. Calibration curve for intact human insulin analysis from charcoal stripped human plasma

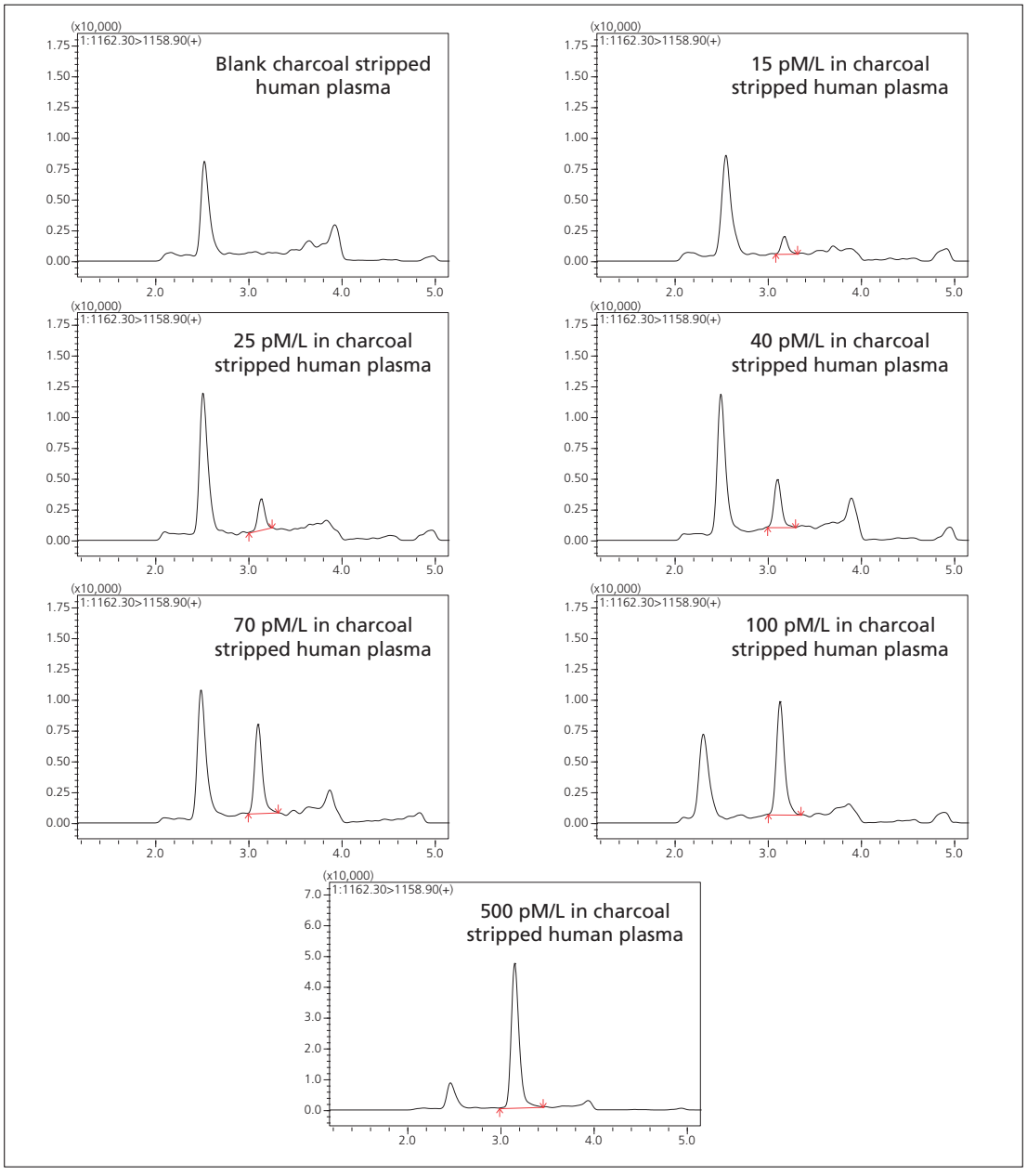

Figure 4. MRM chromatograms of linearity levels for intact human insulin in charcoal stripped human plasma

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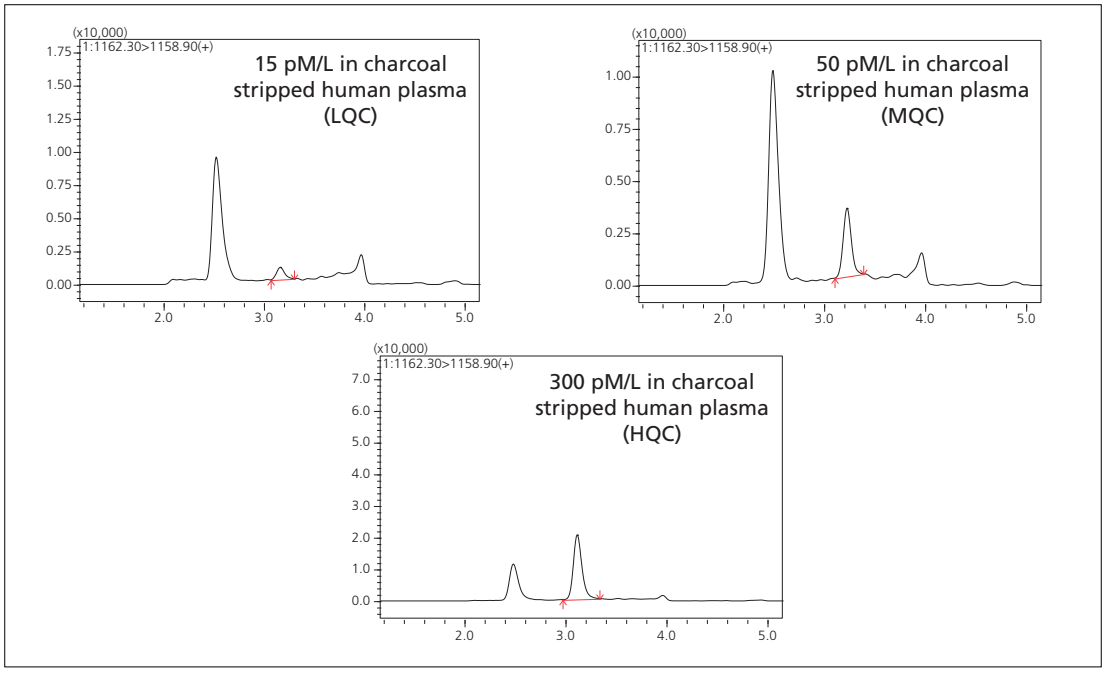

Figure 5. MRM chromatograms of QCs for intact human insulin in charcoal stripped human plasma

Table 2. Results of precision and accuracy for intact human insulin

Name of compound	Standard concentration (pM/L)	Calculated average concentration from calibration graph (pM/L) (n=3)	Average % accuracy (n=3)	% RSD for area counts (n=3)
Intact human insulin	15	15.10	100.67	17.90
	25	25.25	101.00	5.55
	40	38.60	96.50	10.84
	70	71.14	101.63	7.93
	100	100.09	100.10	10.79
	500	509.66	101.93	10.27
	LQC (15)	15.33	102.20	10.28
	MQC (50)	48.89	97.77	13.70
	HQC (300)	252.55	84.17	1.72

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Conclusion

- Simple quantitation workflow was developed for analysis of intact human insulin from charcoal stripped human plasma on LCMS-8060.
- Ultrahigh sensitivity of LCMS-8060 enabled LOQ of 15 pM/L for intact human insulin from charcoal stripped human plasma. Linearity was plotted from 15 to 500 pM/L with r^2 - 0.9968.

References

[1] Yong-Xi Li1, et al., J Anal Bioanal Tech, Volume S5 (2013), 004.
[2] Steven W. Taylor, et al., Clinica Chimica Acta, Volume 455 (2016), 202-208.

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