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Validation of an IC - LC-MS/MS Method for the Quantification of Insulin Aspart in Human Plasma and Serum

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PURPOSE

Insulin Aspart is a rapid-acting insulin analog indicated to improve glycemic control in adults and children with diabetes mellitus. While highly sensitive assays have historically been developed for insulin by immunoassay, a general lack of specificity in the technique has spurred implementation of LC-MS/MS. In this way, closely related insulins can be distinguished with the additional advantages of short development time, multiplexing capability, and enhanced precision and accuracy. More recently, hybrid immunocapture (IC) – LC-MS/MS approaches have leveraged the best of both techniques, illustrated in the current research via development and validation of a highly sensitive and specific method for the determination of Insulin Aspart extracted from human plasma and serum.

OBJECTIVE

To develop and validate a sensitive, specific and high-throughput LC-MS/MS assay to support the PK analysis of Insulin Aspart in adults with type 1 diabetes.

METHODS

Scheme 1. Insulin Aspart Sample Processing

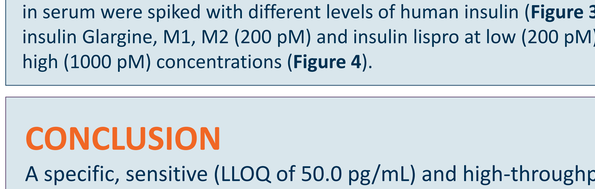
- Plasma/serum (250 µL) + IS (insulin pork) + mAb-conjugated beads (25 µL)
- Incubate at RT for 90 min with shaking
- Process samples on KingFisher™ Flex
- Wash with (1) PBST, (2) PBS, and (3) Elute in 50% MeOH + 1% FA (125 µL)
- Inject 20 µl for LC-MS/MS analysis

LC conditions: Insulin Aspart was separated on a Waters XBridge Protein BEH C4 column (50 x 2.1mm, 3.5 µm) using a 3 min 10% to 50% of 2% TFE + 0.1% formic acid in acetonitrile, gradient elution.

MS conditions: Insulin Aspart (971.80 (6+) > 136.10) and Insulin Pork IS (964.00 (6+) > 136.10) were monitored on a SCIEX Triple Quad API5000.

RESULTS

Figure 1. Representative chromatograms of extracted blank and LLOQ (50.0 pg/mL; 8.58 pM) samples in human plasma (left) and serum (right)



Method Validation

Precision and accuracy of the assay was evaluated by extracting replicate QC samples (N=6) at LLOQ, low, mid and high concentrations on separate days (N=3). For this assay, criteria of accuracy (≤20% expected value except ≤25% at LLOQ) and precision (≤20% CV except ≤25%CV at LLOQ) were used.

The assay is linear (weighted $1/x^2$ regression), precise and accurate within an analytical range of 50.0 to 10000.0 pg/mL (8.58 to 1716.58 pM). Intra- and inter-run precision and accuracy in plasma and serum along with specificity, recovery and stabilities in both matrices are summarized in **Table 1**. Representative chromatograms of blank and LLOQ extracted samples in plasma and serum are shown in **Figure 1**.

Impact of Anti-Insulin Antibody (AIA) on assay

Due to the nature of the immunocapture approach, potential interference from anti-insulin antibody in plasma was evaluated (**Figure 2**). A decrease in analyte and IS response was observed only at the high QC level in the presence of 5-fold molar excess of anti-insulin antibody.

Impact of human insulin and insulin analogs on assay

No significant interference was detected when low and high QC samples in serum were spiked with different levels of human insulin (**Figure 3**), insulin Glargine, M1, M2 (200 pM) and insulin lispro at low (200 pM) and high (1000 pM) concentrations (**Figure 4**).

Figure 2. Impact of Anti-Insulin Ab (AIA) on Assay

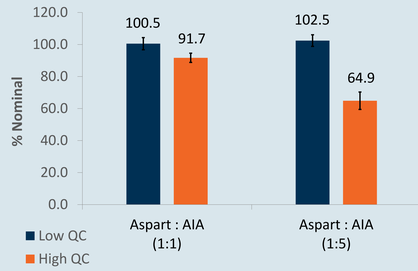


Figure 3. Impact of Human Insulin on Assay

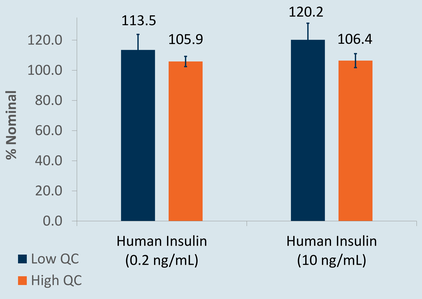


Figure 4. Impact of Insulin Analogs on Assay

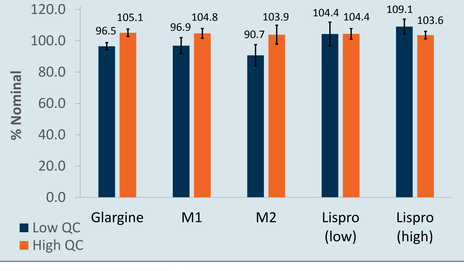


Table 1. Summary of Method Validation Results

Evaluation	Results
Precision and Accuracy (intra-run) in Plasma	LLOQ QC: 104.4%, CV = 9.3% All QC levels: 104.4% - 112.9% CV = 2.6% - 11.7%
Precision and Accuracy (inter-run) in Plasma	LLOQ QC: 109.0%, CV = 10.2% All QC levels: 101.7% - 109.0% CV = 6.2% - 10.2%
Precision and Accuracy (intra-run) in Serum	LLOQ QC: 100.2%, CV = 15.3% All QC levels: 93.1% - 96.8% CV = 3.3% - 8.6%
Precision and Accuracy (inter-run) in Serum	LLOQ QC: 98.5%, CV = 12.1% All QC levels: 97.1% - 98.5% CV = 6.5% - 12.1%
Recovery of Analyte	Plasma: 75.4% to 92.0% (all QC levels) Serum: 81.6% to 90.0% (all QC levels)
Matrix Factor	Acceptable for 10 lots including lipemic and hemolyzed in both matrices
Specificity	Acceptable for 10 lots including lipemic and hemolyzed in both matrices
Autosampler Storage Stability	Plasma: 139.9 hours at 4°C nominal Serum: 116.7 hours at 4°C nominal
Short-Term Stability	Plasma: 22.6 hours at 4°C nominal Serum: 21.0 hours at 4°C nominal
Long-Term Stability	Plasma: 402 days at -20°C and -80°C Serum: 153 days at -20°C and -80°C
Freeze Thaw	4 cycles in both matrices
Whole Blood Stability	Plasma: 2.0 hours in an Ice/Water bath Serum: 1.5 hours at 22°C
Carryover	No significant carryover (≤ 20.0% of LLOQ) observed

CONCLUSION

A specific, sensitive (LLOQ of 50.0 pg/mL) and high-throughput method was developed and fully validated for Insulin Aspart in human plasma and serum, highlighting the advantages of IC-LC/MS methods in leveraging both sensitivity and specificity. This assay is currently used to characterize the pharmacokinetic (PK) profile of Insulin Aspart in patients with T1DM.

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