Quantitation of Insulin Glargine and Major Metabolites in Human Plasma Using Hybrid LBA-LC/MS with Automated Magnetic Particle Processing

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Purpose
Insulin Glargine is a bio-engineered long-acting insulin analogue used to regulate sugar levels in type1 and type2 diabetes. Following subcutaneous injection, Glargine is enzymatically cleaved to generate two active metabolites, M1 and M2. 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 manner, closely related insulins may be distinguished with the additional advantages of shorter development time, multiplexing capability, and enhanced precision and accuracy. Most recently, hybrid LBA-LC/MS approaches have leveraged the best of both techniques, illustrated in the current research via development of a highly sensitive and specific method for the determination of Glargine and M1/M2 metabolites extracted from human plasma.

Methods
Glargine, M1 and M2 were spiked in human plasma from 50 pg/mL – 10,000 pg/mL. Immunoaffinity capture and enrichment of intact insulin Glargine, M1 and M2 was achieved using mouse anti-insulin monoclonal antibody-coated magnetic beads. Sample preparation was performed using 0.5 mL of plasma with porcine insulin as internal standard. Magnetic particle processing including binding, washing and eluting was automated using a Thermo Scientific KingFisher™ Flex. Extracted analytes were separated on a Waters XBridge Protein BEH C4 column (2.1 x 50 mm, 3.5 μm; 0.35 mL/min) with MRM detection of positively formed electrospray ions (Sciex API 5000).

Results
The intact mass analysis approach was used to differentiate Insulin Glargine from its two closely related metabolites. However, the LC-MS/MS determination of intact insulins was notably challenging due to poor fragmentation, non-specific binding and poor solubility. Several experimental factors were optimized to increase assay sensitivity, specificity and robustness, including choice of monoclonal Ab for immunoaffinity capture, the type of magnetic beads used, antibody-to-bead ratio and automation using the KingFisher™ Flex, including wash and elution solutions to allow high throughput analysis of clinical samples. Additionally, the MRM transitions required to achieve requisite sensitivity levels were judiciously selected in lieu of the poor fragmentation characteristics exhibited by Glargine and metabolites. An LLOQ of 50 pg/mL for Glargine, M1 and M2 was established with linearity to 10,000 pg/mL. Intra-assay precision and accuracy for all analytes was 12% and 93% - 104%, respectively, whilst inter-assay precision and accuracy (n = 3) was 11% and 96% - 105%. Recovery, matrix effect, stability, specificity (10 donors, including hemolyzed and lipemic) and anti-drug antibody cross-reactivity were also established. Further, it was determined that with only minor adjustments to the assay, other insulin analogues could be successfully quantitated in human plasma with similar sensitivity, including Lispro, insulin Aspart and insulin Detemir, thus highlighting the general applicability of the LBA-LC/MS hybrid approach for this class of biotherapeutic.

Conclusion
In conclusion, the LBA/LC-MS hybrid approach is ideal for the quantification of insulin Glargine and its active metabolites, in order to leverage both sensitivity and specificity. The specific and enhanced enrichment using immunocapture followed by the multiplexing capabilities of mass spectrometry, makes this approach a universal platform.