ABSTRACT
Quantitation of insulin and insulin analogs has come extremely high importance in supporting of clinical monitoring and drug development in recent years. Due to the complexity of plasma samples, it is important to develop a high-throughput, sensitive, robust, and reproducible method to screen insulin content in plasma samples.

INTRODUCTION
As one of the best known and earliest biopharmaceutics, insulin and its analogs have been extensively used for daily treatment of type I diabetes mellitus. Since the introduction of human insulin (Humulin®), insulin glargine (Figure 1) is a popular long-acting human insulin analogue because of its low absorption to bloodstreams and low side reactions. Quantitation of insulin and insulin analogues traditionally relies on ligands-immunoassay like ELISA or RIA, or suffer from the tedious protein digestion procedures. In this poster, we report a LC-MS/MS based workflow for intact insulin analogue and reduced insulin β chain quantitation. Two different sample preparation procedures were introduced providing high robustness and reproducibility. By developing a SPE procedure, the quantitation result based on transitions 867/984 and 867/992.4. The ULOQ levels are determined based on clinical sample concentration range and LC column performance. More importantly, while a lot of people are suffering with the extreme poor accuracy and reproducibility (Figure 4), our workflow showed impressive quantitation accuracy and reproducibility (Figure 5).

MATERIALS AND METHODS
Sample Preparation
Insulin stock solutions and plasma insulin stock solutions (standard stock) were prepared in 10/30/60 Acetic Acid / MeOH / H2O and PBS + PBST. The plasma samples were prepared in 1:1 with 1/49/50 Acetic Acid / MeOH / H2O. It is reduced by 10 mM DTT, alkylated by 50 mM IAM and subjected to LC-MS/MS analysis. In procedure 2, plasma sample were processed through beads based immunocapture and to achieve the maximum sample recovery. The SPE eluents were diluted with water and directly subjected into LC-MS/MS system. Reduced/digested peptides were subjected to LC-MS/MS analysis. In procedure 2, plasma sample were processed through beads based immunocapture and to achieve the maximum sample recovery. The SPE eluents were diluted with water and directly subjected into LC-MS/MS system. Reduced/digested peptides were subjected to LC-MS/MS analysis. In procedure 1, plasma samples were spiked with insulin Glargine or Insulin (Internal standard) and processed through beads based immunocapture and to achieve the maximum sample recovery. The SPE eluents were diluted with water and directly subjected into LC-MS/MS system. Reduced/digested peptides were subjected to LC-MS/MS analysis.