1. Overview
Glargine and its active metabolites (M1 and M2) were analyzed in human plasma using a solid phase extraction method in LCMS-8060. The developed method is novel, simple and sensitive for simultaneous extraction of glargine and its active metabolites in human plasma.

2. Introduction
Insulin Glargine is a recombinant human insulin analog with long-acting, blood glucose-lowering activity. Insulin glargine differs from human insulin by replacing asparagine with glycine in position 21 of the A-chain and by carboxy-terminal extension of B-chain by 2 arginine residues. After subcutaneous injection, glargine undergoes an enzymatic removal of the basic arginine pair at positions 30B and 31B to yield 21A-threonine to 21A-Gly-des-30B-Thr-human insulin (metabolite 2 [M2]). Insulin glargine undergoes enzymatic removal of the basic arginine pair at positions 30B and 31B to yield 21A-Threonine to 21A-Gly-des-30B-Thr-human insulin (M2) [2].

3. Materials and methods
3.1. Sample preparation
• Preparation of calibration curve standards and quality control (QC) samples
Calibration standards of Insulin glargine, M1 and M2 were prepared in human plasma at concentration levels ranging from 75 to 10000 pg/mL. Quality control samples were prepared at concentration levels between 100 to 2000 pg/mL for glargine and its metabolites (M1 and M2) respectively.
• Sample extraction
SPE eluent was evaporated at 40°C till dryness in nitrogen evaporator. The residue was reconstituted in 100 µL of reconstitution solution, vortexed and filled in HPLC vials for injection.

4. Results
4.1. Selectivity
Selectivity of the method was evaluated by analyzing 6 different lots of blank human plasma and blank plasma spiked with analytes (refer figure 4A, 4B & 4C). The details of LCMS conditions are given in Table 1. Intra-day and inter-day precision and accuracy
Table 1. Instrument parameters for analysis of glargine and its metabolites (M1 and M2)

5. Conclusion
To the best of our knowledge this is the first report of fully validated method which is simple, sensitive and that can rapidly and reliably quantify glargine, M1 and M2 in human plasma. Ultra-high speed and high-sensitivity analysis was achieved on Nexera X2 UHPLC system and has significant drawbacks. This motivated us for this ready to use solutions, we partner with your labs to achieve desired results in your scientific endeavors.

6. References

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