OBJECTIVE
The identification and mitigation of a concentration-dependent interference was necessary to develop and validate an accurate and precise method for the quantitation of HIP2B in human plasma.

INTRODUCTION
Human Proislet Peptide (HIP) is a bioactive peptide that stimulates the differentiation of insulin-producing cells from existing progenitor cells within the pancreas. Neoislet neogenesis is a novel approach to the treatment of both Type 1 and Type 2 diabetes. Pre-clinical studies demonstrated that treatment with HIP2B (a stabilized form of HIP) increased the formation of new glucose- and hormone-responsive β-cells, resulting in attenuation of hyperglycemia. A validated biophysical method was required to characterize the pharmacokinetics of HIP2B in human studies. The validated method was successfully used to measure concentrations of HIP2B in plasma samples from healthy males participating in a safety and tolerability study.

EXPERIMENTAL
HIP2B and its 13C14,15N3-labeled internal standard (IS; +17 amu) were used to measure concentrations of HIP2B in plasma samples from diabetic patients. The validated method was successfully used to measure concentrations of HIP2B in plasma samples from healthy males participating in a safety and tolerability study.

RESULTS
The validated concentration range was 10.0 to 1000 ng/mL. Inter-batch accuracy (10% bias) and precision (CV<10%) for the calibration samples ranged from 0.9 to 3.0 and 0.1 to 9.5, respectively. The mean (n=6) correlation coefficient was 0.9887 ± 0.0026.

IMPLICATIONS
Peptide ions formed during electrospray ionization are highly solvated. Adduct formation results in an overly complex spectrum and loss of sensitivity for the peptide of interest. In this case, the most abundant peptide adduct also produced a quantifiable interference at the mass transition of the stable-labeled internal standard.

CONCLUSION
An accurate and robust method was developed, validated, and employed for the quantitation of HIP2B in clinical samples from a single ascending dose safety and tolerability study.

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