

POSTER 11

**DEVELOPMENT OF HUMAN C-PEPTIDE LC-MS ISOTOPE-DILUTION ASSAY:
OPTIMIZATION OF C-PEPTIDE ISOLATION FROM BIOLOGICAL FLUIDS AND
WITH ION EXCHANGE CHROMATOGRAPHY.**

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Background: Human C-peptide is an effective marker of insulin secretion in diabetes diagnostics, as it is produced in equimolar amounts with insulin. Little metabolism of C-peptide by the liver also enables its concentrations to be three to five times higher than insulin in the plasma. Although there exists a current demand for higher sensitivity, the method of C-peptide quantitative analysis by Isotope-dilution assays allows for greater specificity as compared to current immunoassay methods

Methods: C-peptide isolation from serum was performed by a multi-step procedure (utilizing IDA techniques) of alcohol precipitation, ion exchange chromatography (IEx), and lyophilization. Analysis was done on a LC-MS system consisting of a paired Shimadzu Prominence HPLC system with a Varian Pursuit C18 reverse phase column and API 4000 MS/MS system. IEx was performed on a Hitrap™ SP column. Fragments were monitored in selected ion & multiple reaction monitoring modes.

Results: It was identified that upon passage of C-peptide through ion exchange chromatography, C-peptide levels were amplified, while background was reduced. Post-lyophilization (after IEx as well) displayed the greatest amplification of C-peptide and reduction of background.

Conclusions: The multi-step process utilizing ion exchange chromatography displayed an improvement in optimization of C-peptide isolation compared to standard IDA methods of C-peptide isolation and quantification. High optimization of C-peptide isolation by use of IEx can result in increased sensitivity as well as promote accuracy of calibrators during clinical immunoassays.