

## Challenges and advances in absolute quantitation of peptide hormones

**Eduard Rogatsky**

*Albert Einstein College of Medicine, USA*

### Abstract

The typical method of choice for quantitative LC/MS analysis of small molecules and tryptic digests is MRM (SRM). However, in our practice of routine quantitation of large peptide hormones/biomarkers, we have found that for trace level analytes measurement (e.g. low pg/ml), MRM does not provide satisfactory results compared to SIM. Post-translation modifications, influence of tertiary structure on electrospray ionization and especially different fragmentation pathways of peptides by collision-induced dissociation (which generates many low-abundant fragments) are common factors reducing MRM-based assay sensitivity.

We have taken an alternative approach to MRM based assays, by implementing 2D LC-LC/MS with stable isotope labeled internal standards and have achieved highly reproducible quantitation of low fmole quantities on column for large peptide/small proteins such as human insulin, glucagon, insulin C-peptide and proinsulin.

Using 2D LC for C-peptide analysis we are able to use isotopic labeling to define previously inaccessible aspects of insulin secretion and biosynthesis in vivo. Specifically we have defined a method using deuterated Leucine infusion combined with isotopic analysis of incorporated Leucine into C-peptide for calculation of denovo insulin biosynthesis rates. This method provides a non invasive “biopsy” into the intracellular processes within the insulin producing beta cell. Our novel data is an example of “kinetic proteomics” and has provided fresh insights into the pathophysiology of human diabetes.