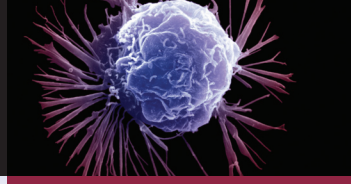

Synthetic Peptides As Internal Standards

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The addition of synthetic internal standards has been used for nearly 40 years for quantitation with mass spectrometry.[1] It is particularly useful with LC-ES-MS and MALDI-MS for proteomic analyses. In the most common workflow for proteomics, peptides are selected based on uniqueness, stability, chromatographic behavior and sensitivity to ionization techniques to serve as surrogate markers for proteins of interest. Internal standards are added to allow these peptides and the proteins they represent to be quantified. The best internal standards are peptides that have identical sequences as the biomarker peptides, and carry stable isotopic labels.[2] The isotopes change the mass, but not the chemical behavior. Isotope ratios of spiked peptides and peptides obtained from protein biomarkers are measured by mass spectrometry and tandem mass spectrometry to provide absolute quantitation of the endogenous protein.[3,4] The use of internal standards allows multiple peptides (and thus multiple proteins) to be quantified in a single sample, and facile quantitation of a particular peptide in multiple samples, *e.g.,* at multiple time points. The approach is applicable to quantitation of proteinsin tissue samples as well as proteins in solution. Peptides with homologous sequences and different masses (and no isotope labels) can also be used as internal standards, supported by standard curves. Isotope-labeled proteins provide even better internal standards because they undergo fractionation and digestion along with the biomarker proteins and provide correction for losses that occur before peptides are produced. Such proteins may be provided from normal or recombinant cells grown in labeled media.

References

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