

Precise Relative Quantification of Proteins in Complex Mixtures Using the Xevo G2 QTof System

GOAL

Quantitative proteomics involves the identification and quantitation of proteins from biological systems. Here we show results from protein mixtures spiked at different concentrations into an *E. coli* cytosolic lysate.

BACKGROUND

Studies of differential protein expression by LC/MS are conducted in many laboratories to gain a better understanding of biological processes. For the identification of proteins, the use of time-of-flight systems is well accepted, and there are advantages in terms of mass accuracy and resolution. However, obtaining good quantitative measurements from complex mixtures with wide dynamic range challenges the entire analytical system from sample preparation to bioinformatics. The label-free LC/MS^E approach avoids the cost and complexity of stable isotope labeling and provides a robust method for making quantitative measurements. In this technical brief we demonstrate the relative quantification of known standard proteins contained in a four protein mixture spiked at two different levels into a complex sample matrix.

Xevo G2 QTof provides quantitative measurements of proteins from complex mixtures with wide dynamic range.

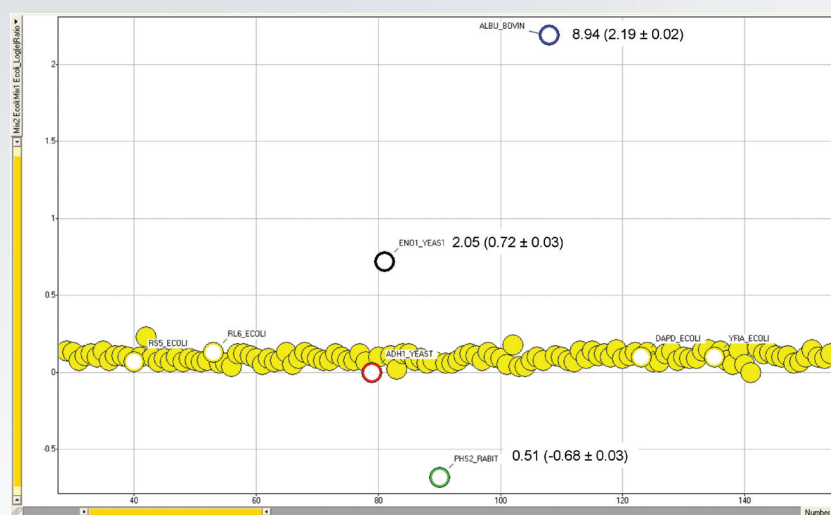


Figure 1. Experimentally determined ratios of the four standard proteins analyzed in the *E. coli* mixture (only a section of *E. coli* proteins is shown).

Protein	Mix 2/Mix 1
P00330 ADH	1.0
P00389 PhosB	0.5
P00924 Enolase	2.0
P02769 BSA	8.0

Table 1. Expected ratio amounts for the four MPDS proteins (+/- 5%).

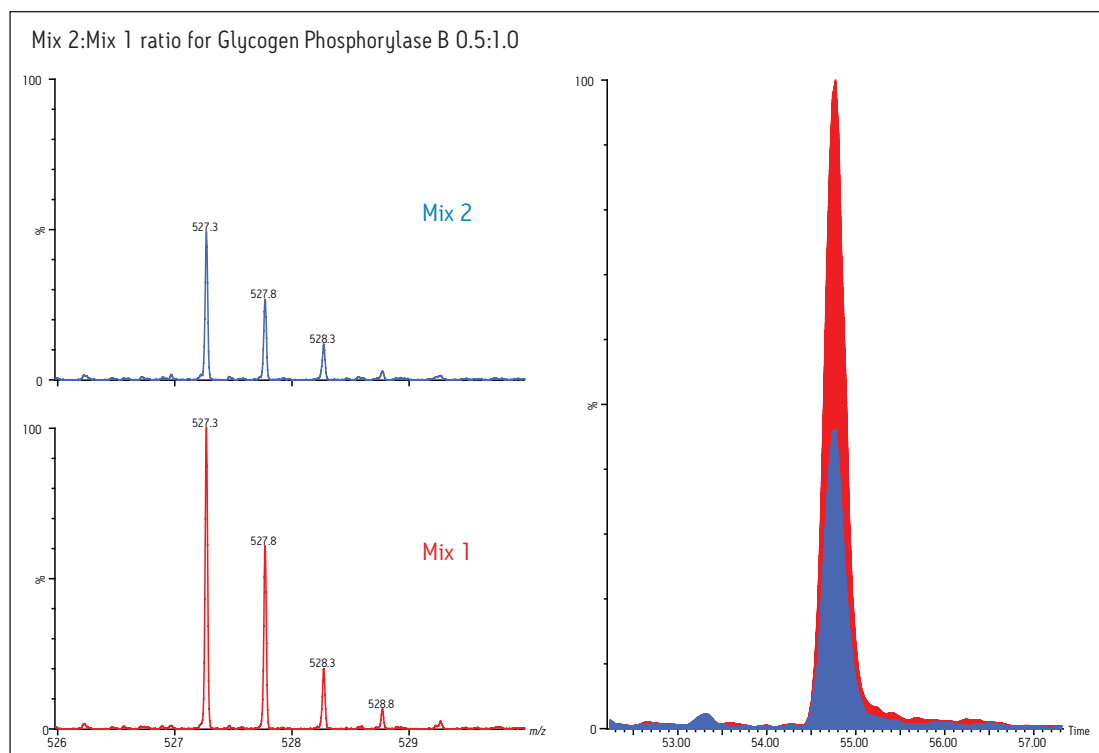


Figure 2. Spectrum at peak top (left) and chromatogram (± 10 ppm) of the $[M+2H]^{2+}$ ion for the VIFLENYR peptide from Glycogen Phosphorylase B at m/z 527.3, which could be determined at a retention time of approximately 54.7 min in both Mix 1 and Mix 2. Vertical axes are linked to show relative intensities.

THE SOLUTION

Standard protein mixtures containing the four proteins, as shown in Table 1, were spiked at an ADH concentration of 25 fmol into a 400 ng sample of digested *E. coli* cell lysate. A Waters® nanoACQUITY UPLC® System coupled with a Xevo™ G2 QTof were used to separate and analyze the tryptic peptides in triplicate. The Xevo G2 QTof was operated in LC/MS^E mode whereby the collision energy was switched between low and elevated energy during alternate scans. Peptide precursor information was acquired in the first function (used for quantitation) and fragment ion information was acquired in the second function (used for protein identification). Using ProteinLynx Global SERVER™ data were processed and searched against a non-redundant *E. coli* database, with the four known protein sequences added. By selecting ADH as the internal standard, relative ratios for each spiked protein were determined.

SUMMARY

Here we have shown relative quantification of four standard proteins spiked into a complex matrix using the Xevo G2 QTof Mass Spectrometer. Good correlation was achieved between the expected and experimentally determined values.

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