An alternative method of quantification is illustrated in Figure 3, where the sum of the intensities of three peptides from alpha gal A, which repli-
cated across all conditions is divided by the sum of the intensities of three peptide of human serum albumin or gamma 1 chain and is dis-
payed as a function of the amount of alpha gal A injected on column.

Figure 3. Relative quantification of alpha galactosidase spikes in unde-
pliceted human serum. Quantification was performed by using summed intensities of identified peptides from the low-energy data set that replicate across all conditions.

Relative quantitation at the peptide level

Identification of proteins can also be conducted from the peptide exact mass only. In this instance, the data sets are clustered based on their exact mass and retention time (EMRT). An example is shown in the up-
per part of Figure 4, where the log intensity ratio is depicted as a func-
tion of mass. A selector tool is used to select the peptides of interest which can be followed by a peptide mass fingerprint type search using the peptide mass information, illustrated in the bottom pane of Figure 4.

Figure 4. Exact mass retention time browser—upper pane: Showing the log intensity ratio of 10 fmol of alpha galactosidase vs. 500 fmol injected on column and the low energy peptide mass fingerprint search result for 10 fmol of alpha galactosidase—bottom pane. Alternatively, the log intensities of the matching peptides between two conditions can be plotted as a function of each other. Figure 5, shows a scatter plot of the human serum containing 50 fmol vs. that with 100 fmol of alpha gal A injected on column. Statistical analysis was con-
ducted to identify statistically significant expression differences.

Figure 5. Average peptide intensity ratio for duplicate injections for con-
dition one (five = 0 hr) vs. condition two (five = 2 hr). Significant inten-
sity changes determined by a student t test and coefficient of variation change in intensity were evaluated and are colored according to the legend above.

CONCLUSIONS

• The data presented showed the feasibility of monitoring the concentration changes of therapeutic proteins in complex serum samples over time.
• The results indicate good limits of detection (10fmol on col-


Figure 6. Shows the identified acute phase reactant proteins during alpha galactosidase infusion over time (12 hrs) plotted by expression ratio and probability as a function of accession number for condition one (five = 0 hr) vs. condition six (five = 12.25 hr).

Figure 7. Acute phase plasma protein response as a function of time, il-


Figure 8. Average peptide intensity ratio for triplicate injections for con-
dition one (five = 0 hr) vs. condition two (five = 2 hr). Significant inten-
sity changes determined by a student t test and coefficient of variation change in intensity were evaluated and are colored according to the legend above.

REFERENCES