A decrease in antibody productivity, with no corresponding decrease in cell growth, is associated with lot-to-lot variability of this hydrolysate. A wheat gluten hydrolysate from Sigma-Aldrich, lot MS, has been used in our laboratory for several years with consistently high cell productivity. The mass assignments for Component 1 and Component 2 are within the mass accuracy specifications of the LCT instrument (± 2 ppm). Figure 7 shows an exact mass overlay of the mass assignments for Component 1 and Component 2. The exact mass assignment for Component 1 was 512.0528 (m/z 512.0554). The exact mass assignment for Component 2 was 490.2862 (m/z 490.2865).

**Abstract**

Secondary Chromatography (Phosphate Separation Method): Mobile phase A was 25 mM Sodium phosphate buffer, pH 6. Mobile phase B was acetonitrile. Separation of the tryptic hydrolysate components was performed using a 21 minute linear gradient from 0 to 7% B. The flow rate was 0.7 ml per minute and the column temperature was maintained at 25°C. The injection volume was 100 µl. The UV and MS data were obtained as described above.

A Chinese Hamster Ovary (CHO) cell line expressing a proprietary recombinant antibody was cultured in CHO Protein Grow Animal Component-Free Medium (Sigma C5467) supplemented with 2.5 g/l of wheat gluten hydrolysate. The cultures, in 100 mL T-flasks, were kept at 37 °C in a humidified atmosphere containing 5% CO2. Quantification of the recombinant antibody was performed by affinity HPLC using a Protein G column from Applied Biosystems.

**Results and Discussion**

Wheat gluten hydrolysate is a complex mixture of polypeptides, primarily rich in stable glutamine. Because the spectral range from 210 nm to 285 nm was collected, and compared in component-wise with the UV absorbance of the hydrolysate samples. The UV spectra of proteins and peptides are very similar and are dependent on the spectra of the amino acids of which they are composed. The absorbance at 280 nm is often used for protein and peptide detection because of the high content of aromatic amino acids. Figure 8 shows a portion of the 280 nm chromatograms extracted ion chromatograms for Component 1 and Component 2. The mass assignments for Component 1 and Component 2 are within the mass accuracy specifications of the LCT instrument (± 2 ppm). The exact mass assignment for Component 1 was 512.0528 (m/z 512.0554). The exact mass assignment for Component 2 was 490.2862 (m/z 490.2865). 

Since the components of interest were now isolated from the non-automorphic components in the TFA fractions, we were able to obtain photodiode array (PDA) spectra and accurate mass spectra of Components 1 and 2 using the phosphate separation. The chromatographic peaks for Component 1 and Component 2 demonstrated good peak purity by PDA and MS analysis. The exact mass of the peak at 11.07 minutes in Figure 4A was determined to be m/z 512.0558 (512.0554). The exact mass of the peak at 17.00 minutes in Figure 4B was determined to be m/z 490.2554 (490.2552). 

**Materials and Methods**

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**Results and Discussion**

Wheat gluten hydrolysate is an enzymatic digest of wheat that provides a high quality source of peptides, which is particularly rich in stable glutamine. During the course of serum-free medium development for CHO cells, the incorporation of this wheat gluten hydrolysate in cell culture media was found to have a positive effect on cell proliferation versus no supplementation with the hydrolysate (Figure 1A). This effect was reproduced in many other CHO cell culture experiments in which a 2.5 g/l wheat gluten hydrolysate was found to be optimal. Several lots of this hydrolysate were analyzed in experiments designed to further demonstrate a correlation between cell culture productivity and the presence of this hydrolysate. These experiments showed that different lots A, B and B1 of wheat gluten hydrolysate produced different degrees of antibody production (Figure 1B).

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