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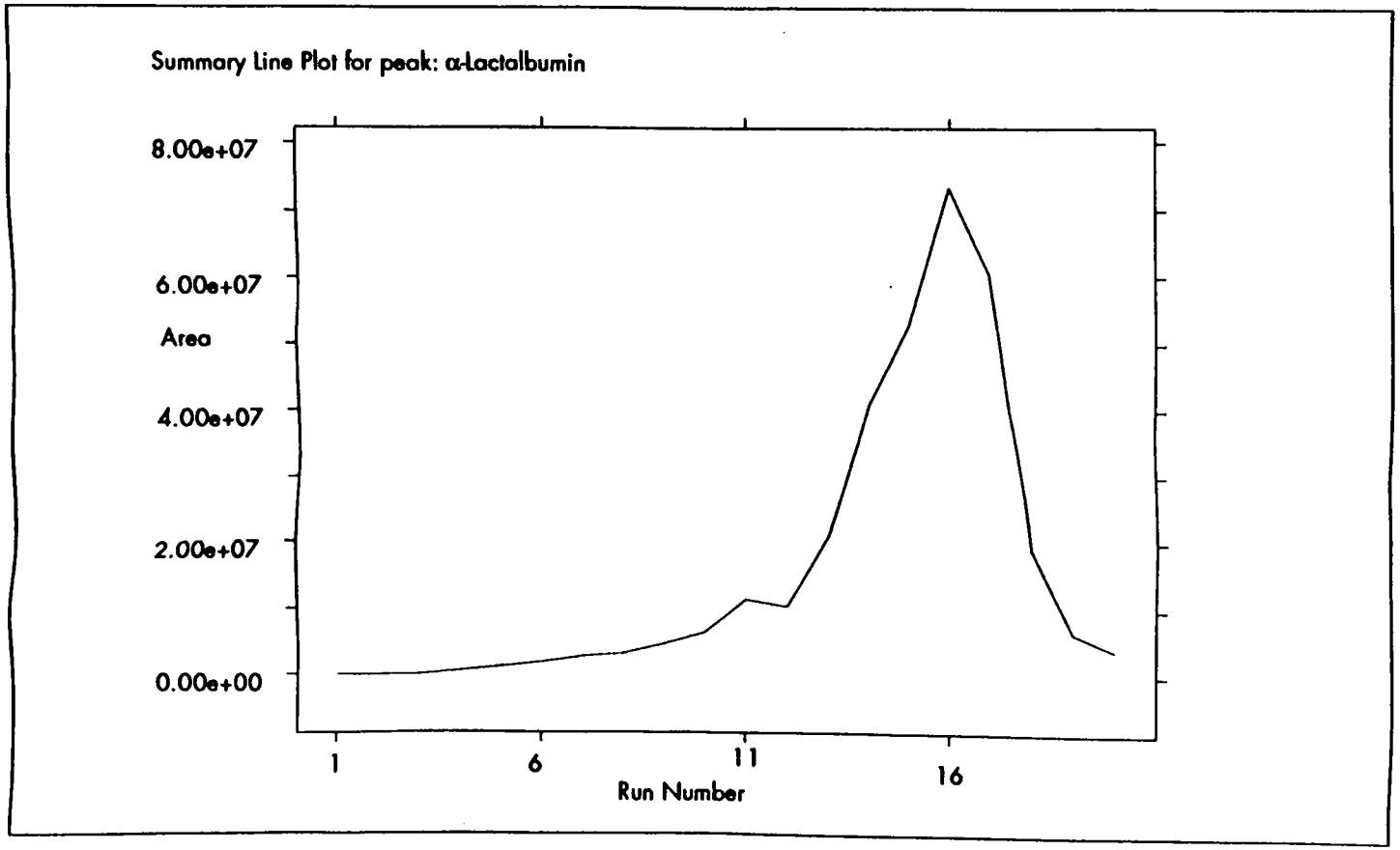
Protein Purification Using System Suitability for Fractional Trending



System suitability can be used for fractional trending during protein purification to rapidly determine which fractions contain the bulk of the protein of interest. In this case, 20 fractions were collected and analyzed using gel filtration HPLC.

Conditions

Column: Shodex™ Protein KW-802.5, 803 and 804

Eluent: 50 mM Sodium phosphate, pH 7.0 with 300 mM Sodium chloride

Flow Rate: 0.5 ml/min.

Detection: 280 nm

Sample: Human Milk Whey

Protein of Interest: α–Lactalbumin

Objective:

To demonstrate the advantage the 845/860 system suitability package provides when purifying proteins and determining fractional content.

Historically, protein purification involved the collection of many fractions from a primary HPLC or FPLC run. All collected fractions were then re-analyzed to determine each fraction's purity and bulk protein content by reviewing each resulting chromatogram. Results were then manually tabulated to determine which fractions could be combined for further purification if required.

Using system suitability, the chemist can automatically generate a summary plot of the peak areas for the protein of interest (as shown on the front) directly from the collected HPLC data. From this single report, the user can easily identify the fractions containing the bulk of the isolated protein. By eliminating the manual data manipulation step, the time involved in the purification process is greatly reduced.

Details:

System suitability is available for use with the VAX based 845/860 ExpertEase system. It is an optional application that when purchased (PN 200233) appears under the "W" menu off the main ExpertEase menu.

Primary purification of milk whey derived α-lactalbumin was performed using reverse phase chromatography on a Delta-Pak C_{18} 15 µm 300 Å column (see reference #2 for more information on the specific conditions). Collected fractions were analyzed repetitively using gel filtration chromatography. Molecular weight of α-lactalbumin is 14,100 daltons.

System:

The System used consisted of a 600E Multi Solvent Delivery System with a 484 Tunable UV/Vis Detector. Data was collected using an 860 ExpertEase Data System (software version 2.3).

References:

C. Phoebe, Jr., K. O' Connor and G. Vella, "The Purification of Human Milk Whey Proteins and Peptides by HPLC", Poster presentation, FASEB/ASBMB, April, 1991, Atlanta.

G. Vella, K. O'Connor, L. M. Jordan, C. Phoebe, Jr., "The Analysis and Purification of Human Milk Whey Proteins by a Multi-Chromatographic Mode Approach", Poster presentation, ASBMB/AAI, June, 1990, New Orleans.

D. Dion, M. Andrews and L. M. Jordan, "Practical Applications of System Suitability Software", Poster presentation, Pittcon '91, Chicago.