

Analysis of Hydrolyzed Bovine Serum Albumin Using Waters AccQ•Tag[™] Method for Amino Acid Analysis

Objective:

This application note shows high resolution, high sensitivity analysis of a hydrolyzed protein sample using Waters AccQ•Tag Method for amino acid analysis. Compositional analysis with sub-microgram sample amounts is demonstrated.

Details:

The AccQ•Tag Method for hydrolysate amino acid analysis uses the novel reagent 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AccQ•Fluor[™] reagent, AQC) to derivatize primary and secondary amino acids in protein and peptide hydrolysates. The reaction yields stable, fluorescent products that can be injected without any further sample preparation. High sensitivity fluorescence detection makes it possible to obtain accurate compositional analysis with sub-microgram sample amounts.



Analysis of derivatized protein hydrolysate samples is simple and accurate with Waters new AccQ•Tag Method.

The table below gives some representative data obtained from hydrolysates of bovine serum albumin. The amounts given are the total sample hydrolyzed; an aliquot of the sample (5% for the 1.3µg sample, 20% for the 30ng sample) was then used for chromatographic analysis. The fitted composition and the error analysis were calculated with the procedure² used by the Amino Acid Analysis Subcommittee of the Association of Biomolecular Resource Facilities.

Amino Acid	Actual Composition	High Level Sample (1.3 µg)	Low Level Sample (30 ng)
Asp	54	58.22	53.72
Ser	28	24.70	29.55
Glu	79	83.78	73.65
Gly	16	15.84	22.91
His	17	16.57	13.75
Arg	23	23.55	23.02
Thr	34	33.03	31.39
Ala	46	47.41	56.13
Pro	28	27.82	28.18
Tyr	19	17.82	16.15
Val	36	35.95	34.48
Met	4	4.06	4.58
Lys	59	60.75	52.00
Lle	14	13.75	14.32
Leu	61	61.48	54.75
Phe	27	27.61	25.77
Average % Error		3.4	10.5

System:

Waters AccQ•Tag System consisting of a 625 LC system equipped with a column heater, 717 plus Autosampler with heater/chiller, and 470 Scanning Fluorescence Detector. System control and results management were provided by a Millennium[™] 2010 Chromatography Manager using the AccQ•Tag Application Template.

References:

1. Cohen, S. A. and Michaud, D. M,. Analytical Biochemistry (1993) in press.

2. Strydom, D. J., Tarr, G. E., Pan, Y-C,E., and Paxton, R. J., Techniques in Protein Chemistry III (R. H. Angeletti ed.) Academic Press, 1992, pp 261-274.



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