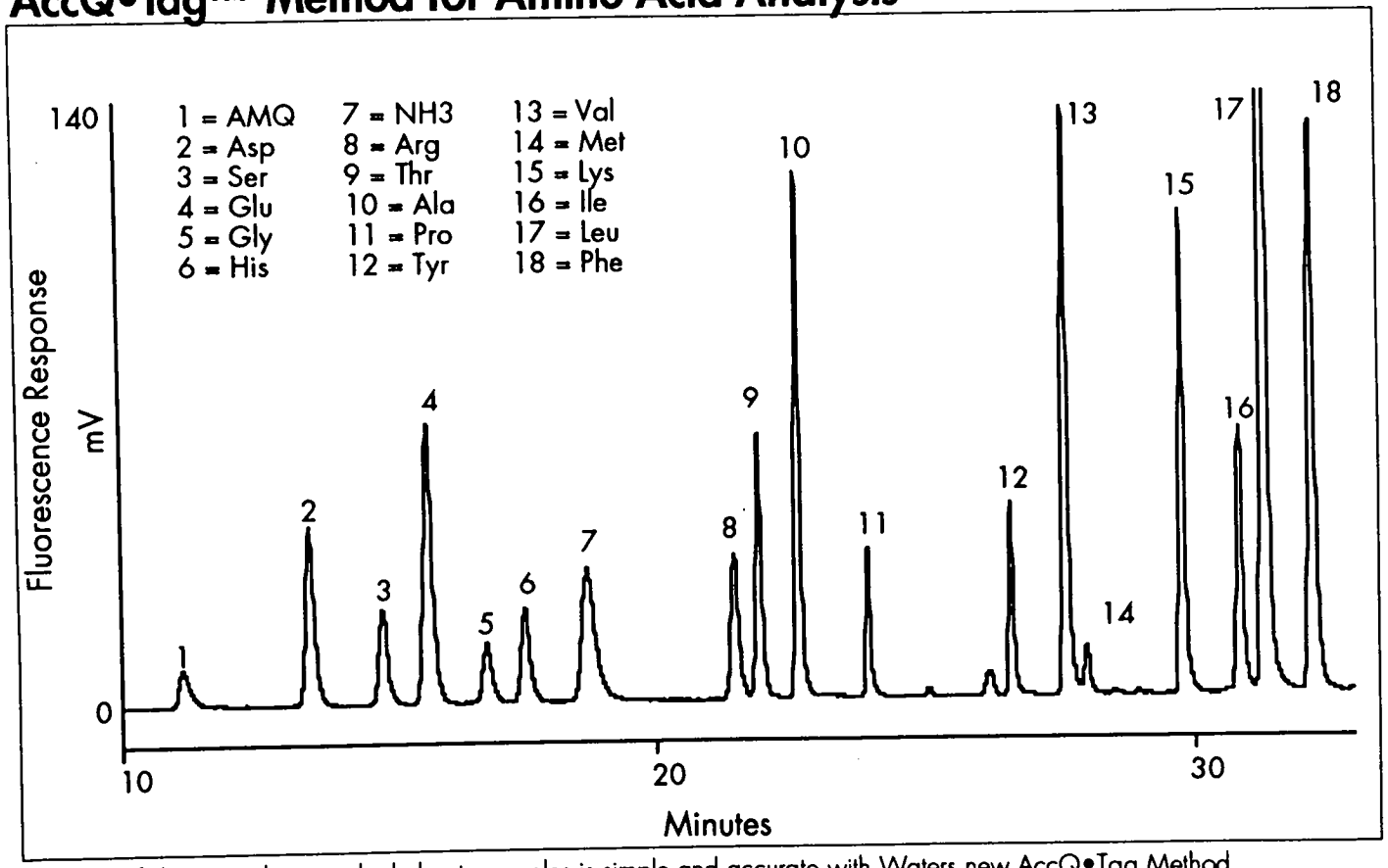
930495

[redacted]

Steven A. Cohen
041

Waters Application Notebook

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



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

Conditions:

Column: Waters AccQ•Tag Column, 3.9 mm X150 mm

Column Temperature: 37°C

Mobile Phase: A ternary gradient elution profile using
Eluent A: AccQ•Tag Eluent A
Eluent B: Acetonitrile
Eluent C: MilliQ® water

Flow Rate: 1.0 ml/min

Detection: 470 Scanning Fluorescence Detector (5µl flow cell)

EX: 250nm
EM: 395nm
Filter: 0.5
Gain: 100

Injection volume: 5µl

Sample: AQC-Derivatized Bovine Serum Albumin Hydrolysate, 1.3µg hydrolyzed, 5% analyzed.

Millipore Corporation Waters Chromatography Division 34 Maple Street Milford Massachusetts 01757 508 478 2000

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.

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 procedure[2] 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
Ile	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 625LC system equipped with a column heater, 717plus 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.

R101