

Determination of Cysteine/Cystine in Protein and Peptide Samples

Application Note #1

Cysteine/Cystine

Of the 20 or so amino acids found in proteins, some are destroyed or modified during hydrolysis. Under normal hydrolysis conditions, cysteine/cystine frequently decompose making quantitation of this amino acid difficult. Oxidation of cysteine/cystine to cysteic acid is a common approach to form a hydrolysis-stable derivative for amino acid analysis. The hydrophilic nature of cysteic acid causes the PTC-derivative to elute as a broad peak near the chromatographic solvent front making accurate quantitation difficult. Improvements in the peak shape and retention of PTC-cysteic acid on the Model 421 Amino Acid Analyzer (Fig. 1) is accomplished by ion-pairing the derivative with diisopropylethylamine using a modified derivatization cycle (Table 1).

Cysteine/cystine are quantitatively converted to cysteic acid during hydrolysis in the presence of DMSO¹. Approximately 0.2 µg (12 pmols) of β-lactoglobulin was vapor phase hydrolyzed in triplicate using 6N HCl with 2% DMSO and analyzed using the Model 421 Amino Acid Analyzer (Fig. 2). The amount of cysteine/cystine was quantitated as cysteic acid. The results below show excellent correlation with the known amount of cysteine/cystine in β-lactoglobulin.

	Lit	
Cysteine/Cystine	Value	Det
Hydrolysis-1	5	4.8
Hydrolysis-2	5	4.4
Hydrolysis-3	5	4.4
Avg		4.5

Key Words:

- Cysteic Acid
- Cysteine/Cystine

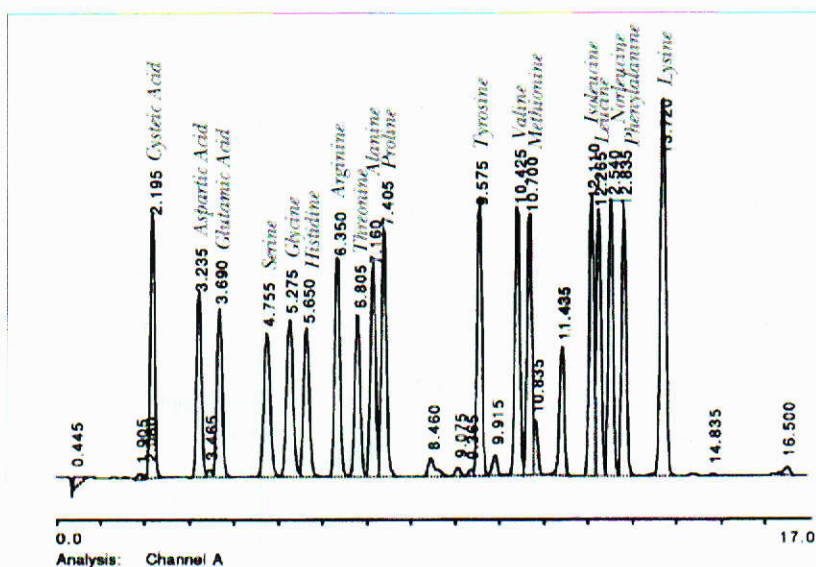


Fig. 1. Analysis of a 250 pmol amino acid standard containing 250 pmol of cysteic acid using the Model 421 Amino Acid Analyzer.

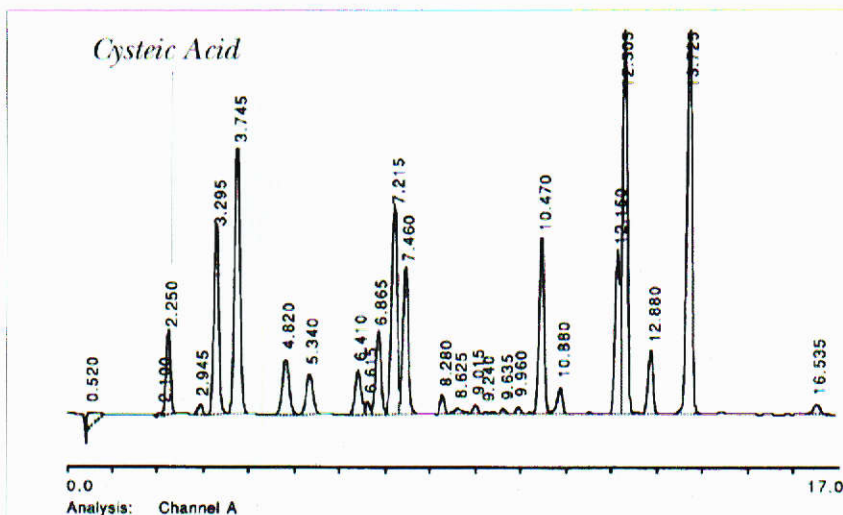


Fig 2. Analysis of 0.2 µg (12 pmol) of β-lactoglobulin on the Model 421 Amino Acid Analyzer after vapor phase oxidative hydrolysis at 150 °C for 40 min. using 6N HCl with 2% DMSO.

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Step #	Fxn X	Description	Time
1	5	DELIVER #3	240
2	19	RELAY 1 ON	1
3	15	FLSH #3, #4	15
4	7	PRESS #3, #4	30
5	1	DELIVER #4	1
6	9	DRV ARGON	90
7	5	DELIVER #3	30
8	13	FLUSH #2	5
9	6	PRESS #2	5
10	4	DELIVER #2	1
11	9	DRV ARGON	180
12	7	PRESS #3, #4	30
13	1	DELIVER #4	1
14	5	DELIVER #3	600
15	9	DRV ARGON	240
16	28	TF TO SEP	60
17	34	ARG TO SEP	180
18	11	FLSH #5, #6	10
19	8	PRES #5, #6	30
20	21	DSOLV SMPL	2
21	22	ARG TO TF	1
22	17	DRV WAIT	30
23	22	ARG TO TF	15
24	21	DSOLV SMPL	3
25	22	ARG TO TF	1
26	17	DRV WAIT	30
27	22	ARG TO TF	15
28	33	DIEA TO TF	180
29	28	TF TO SEP	70*
30	19	RELAY 1 ON	1
31	17	DRV WAIT	10
32	3	DELIVER #6	6
33	9	DRV ARGON	30
34	29	#6 TO TF	6
35	22	ARG TO TF	30
36	23	DRY TF	10
37	28	TF TO SEP	45
38	29	#6 TO TF	6
39	22	ARG TO TF	15
40	23	DRY TF	10
41	28	TF TO SEP	150
42	32	DRV VENT	20
43	18	NEW SAMPLE	0
44	81	CYCLE END	0

Table 1. Cysteic acid derivatization cycle.
(*Input transfer time from standard derivatization cycle.)

Reference

1. Spencer, S., L., and Wold, F., 1969,
Anal Biochem., 32, p. 185-189.