Application Note

Date: No. 430015H-E

Amino acid composition analysis of protein using precolumn derivatization with DABS-Cl

Amino acid composition of protein was analyzed by DAB-Label which is a kit for pre-column derivatization amino acid analysis with DABS-Cl (Dimethylamino azobenzene-sulfonyl chloride).

1 µg of Myoglobin was vapor-phase hydrolyzed with hydrochloric acid (6N HCl, 110 ˚C, 24 hrs) and derivatized with DABS-Cl. The analysis of the derivatives was performed by HPLC. Fig. 1 shows the chromatogram and Table 1 shows the comparison with the theoretical values. It was shown that the amino acid composition obtained by this method was in good agreement with tthe theoretical values.

Keywords: 1. Amino acid, 2. Myoglobin, 3. ODS, 4. Vis, 5. DABS-CI

Conditions:

Column:	Dabsylpak		
Precolumn:	Dabsylpak-P		
Eluent A:	50mM NaH2PO4(pH6.6)/DMF(96/4)		
Eluent B:	CH3CN		
Time(min.)	A(%)	B(%)	1Cycle 65.0min
0.0	85	15	
18.0	70	30	
28.0	59	41	
32.0	49	51	
36.0	46	54	
44.0	36	64	
46.0	10	90	
48.0	10	90	
50.0	85	15	
Wave length	: 465nm		
Flow rate	: 1.0ml/min		
Column Temp	: 40 degree celsius		
Sample	: Myoglobin		
Injection volume	: 20ul		

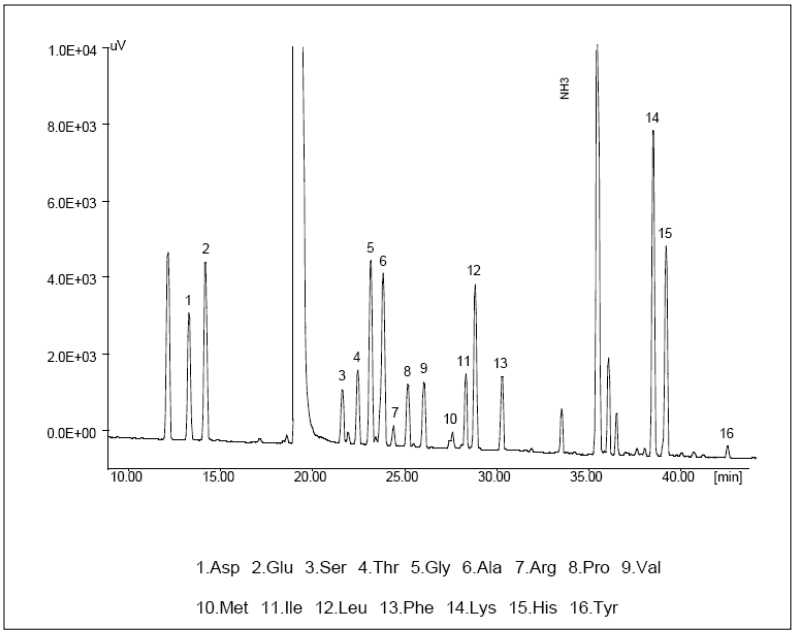

Fig.1 Chromatogram of hydrolyzed myoglobin

Amino acids	n=1	n=2	Theoretical values
Arg	9.3	9.9	10
Glu	17.1	18.5	19
Ser	4.1	4.6	5
Thr	6.5	7.2	7
Gly	12.1	12.8	15
Ala	13.1	14.0	15
Arg	2.0	2.0	2
Pro	4.0	4.1	4
Val	5.2	5.9	7
Met	1.3	1.3	2
Ille	5.9	7.3	9
Leu	14.0	15.4	17
Phe	5.5	5.8	7
Lys	15.4	16.4	19
His	9.5	10.0	11
Tyr	1.5	1.5	2

Table 1 Comparison with theoretical values

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