# Application Note

440013H

## High Resolution Analysis of Tryptic Digests of Bovine Serum Albumin by Ultra High-performance Liquid Chromatography with Photodiode Array Detection

### Introduction

The peptide mapping is one of the testing methods for biomedicine. This method requires HPLC or other separation analysis of peptide segments digested by enzymes or chemicals, and it is one of the internationally harmonized method among the U.S. Pharmacopoeia (USP), the European Pharmacopoeia (EP) and the Japanese Pharmacopoeia (JP). In this method, the standard protein is digested in the same manner as in the target protein in order to directly compare the peptide mapping of standard protein with that of the target protein so that the expression of recombinant proteins can be detected. The method is applied to quality assurance of various food products.

In this report, the peptide mapping of tryptic digests of BSA by using of X-LC system is demonstrated.

**Key word:** peptide mapping, BSA,tryptic digestion, 1.8 µm, C18 column, PDA detector

### Experimental

| Equipment. | | Conditions. | |
|---|---|---|---|
| Pump: | X-LC 3085PU x 2 | Column: | ZORBAX SB-C18 (2.1 mmID x 150 mmL, 1.8 µm) |
| Degasser: | X-LC 3080DG | Trapcolumn: | ODS (2.0 mmID x 10 mmL, 5 µm) |
| Dynamic Mixer: | X-LC 3180MX | Eluent A: | 0.05% Formic acid/Acetonitrile (97/3) |
| Column oven: | X-LC 3067CO | Eluent B: | 0.05% Formic acid/Acetonitrile (40/60) |
| Autosampler: | X-LC 3159AS | Gradient condition: | (A/B), 0 min(100/0)→10, 60, 120 min (20/80) |
| Detector: | X-LC 3110MD | Flow rate: | 0.2 mL/min |
| | | Column temp.: | 40ºC |
| | | Wavelength: | 215 nm |
| | | Injection volume: | 5 µL |
| | | Standard sample: | 1.2 µg/µL BSA tryptic digest |

### Results

Chromatograms of tryptic digests are shown in Fig. 1. Depending on the gradient profiles, the number of eluted peak varies: 93 peaks in 10 min gradient, 139 peaks in 60 min gradient and 125 peaks in 120 min gradient.

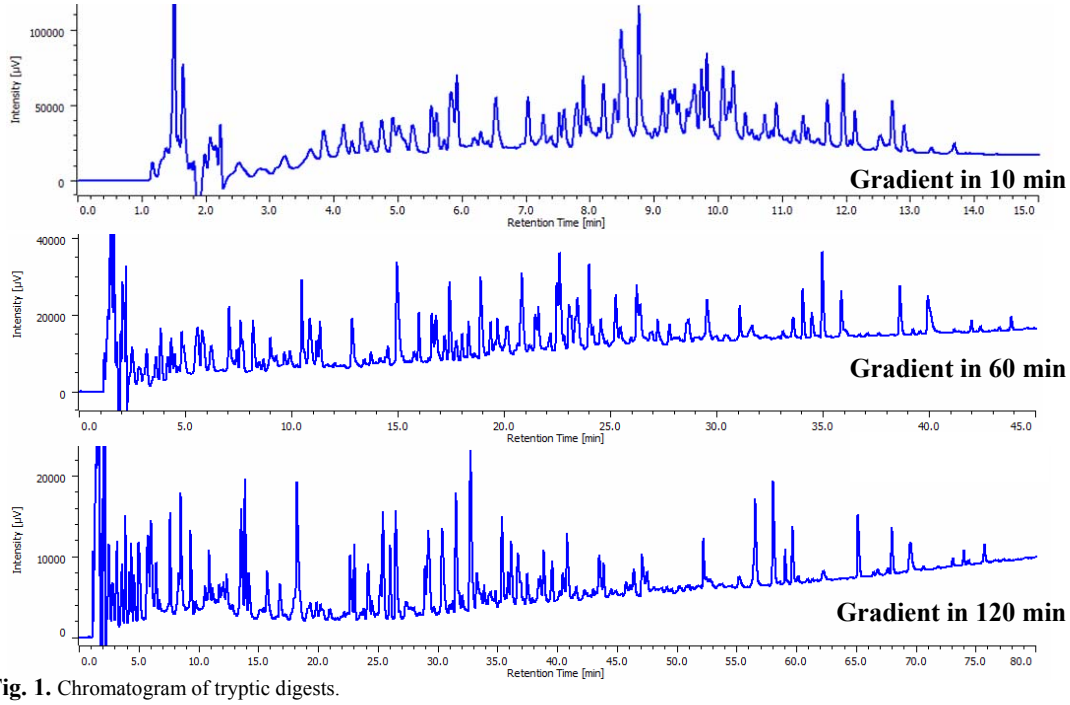


**Fig. 1.** Chromatogram of tryptic digests.

Sample preparation. 1) Dissolve 10 mg of BSA in 1.5 mL of solvent (6 M Urea: 0.1M NH4HCO3=1:4). 2) Add 200 µl of 1% of trypsin in 0.003N HCl to BSA solution so that the ratio of BSA solution and 1% trypsin becomes 5:1 (w/w). 3) Digest the stand at 37ºC for 15 hours. 4) Perform ultrafiltration by model Ultrafree C3 UFC3LGC00 (10,000MWCO) membrane. 5) Dilute 5 times with mobile phase A.



**JASCO INTERNATIONAL CO., LTD**

4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512