



## High-Resolution Analysis of Tryptic Digests of Bovine Serum Albumin using UHPLC with Photodiode Array Detection

### Introduction

Peptide mapping is a standard testing method for biomedicines. This method requires HPLC separation of peptide segments digested using enzymes or chemicals and is an internationally harmonized method described by the U.S. Pharmacopoeia (USP), the European Pharmacopoeia (EP) and the Japanese Pharmacopoeia (JP).

The target protein and a standard protein are digested using the same protocol. A direct comparison of the peptide map of the target protein can be made with the standard protein so that the expression of recombinant proteins can be assessed. This method is applied to the quality assurance of a variety of food products.



Jasco XLC-3000

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## Experimental

### XLC-3000 System

Pump:	X-LC3085PU x 2
Degasser:	X-LC3080DG
Dynamic Mixer:	X-LC3180MX
Column oven:	X-LC3067CO
Autosampler:	X-LC3159AS
Detector:	X-LC3110MD

### Conditions

Column:	ZORBAX SB-C18 (2.1mmIDx150mmL, 1.8µm)
Trapcolumn:	ODS (2.0mmIDx10 mmL, 5 µm)
EluentA:	0.05% Formicacid / Acetonitrile (97/3)
EluentB:	0.05% Formicacid / Acetonitrile (40/60)
Gradient condition:	(A/B), 0min (100/0) 0, 60, 120min (20/80)
Flowrate:	0.2mL/min
Column Temperature.:	40°C
Wavelength:	215nm
Injection Volume:	5µL
Standard Sample:	1.2µg/µL BSA trypticdigest

## Sample Preparation

- 1) Dissolve 10 mg of BSA in 1.5 mL of solvent (6 M Urea: 0.1M NH<sub>4</sub>HCO<sub>3</sub>=1:4).
- 2) Add 200 µl of 1% of trypsin in 0.003N HCl to BSA solution to a ratio of BSA solution to 1% trypsin at 5:1 (w/w).
- 3) Digest and incubate at 37°C for 15 hours.
- 4) Perform ultrafiltration using model Ultrafree C3 UFC3LGC00 (10,000MWCO) membrane.
- 5) Dilute 5 times with mobile phase A.

## Results

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

**Figure 1. Chromatogram of tryptic digests.**

