A UHPLC method to improve peak capacity of peptide mapping of digestion of factor IX

Application Note

Drug Development and QA/QC

Author

Yao Xiao, Vincent

Agilent Technologies

Beijing, China

Abstract

UHPLC with sub-2-µm (STM) technology is the most powerful method for improving speed, separation and peak capacity of LC analyses. The Agilent 1290 Infinity LC system has a broad power range, and exhibits significant compatibility for both HPLC and UHPLC applications.

This Application Note describes a UHPLC method for analyzing the human factor IX with the Agilent 1290 Infinity LC system and an Agilent ZORBAX SB C8 RRHD column, which is widely used for peptide analysis. The analysis time was less than 30 minutes, and the method employed a 100 mm column to match the gradient for a high peak capacity.

Human factor IX is a glycoprotein used to cure hemophilia B. RP-HPLC methods are widely used to evaluate these products. In this method the protein is first hydrolyzed to peptides and then analyzed by the HPLC. The peptide fingerprint chromatography is evaluated as the quality control indicator. However, the HPLC method requires over two hours to analyze a sample. The peak capacity is low and most peaks are broad because of low flow rates. In addition the separation is poor.

In this study, the UHPLC method using the Agilent 1290 Infinity LC system optimized separation. A 100 mm column was used to achieve good separation and peak capacity. A high flow rate gained larger peak capacity, though the highest pressure was 515 bar. However, the peak shapes tailed when larger flow rates were used. The temperature was also optimized to ensure good peak shape. The temperature of the method was higher than usual for peptide separation.

Agilent Technologies

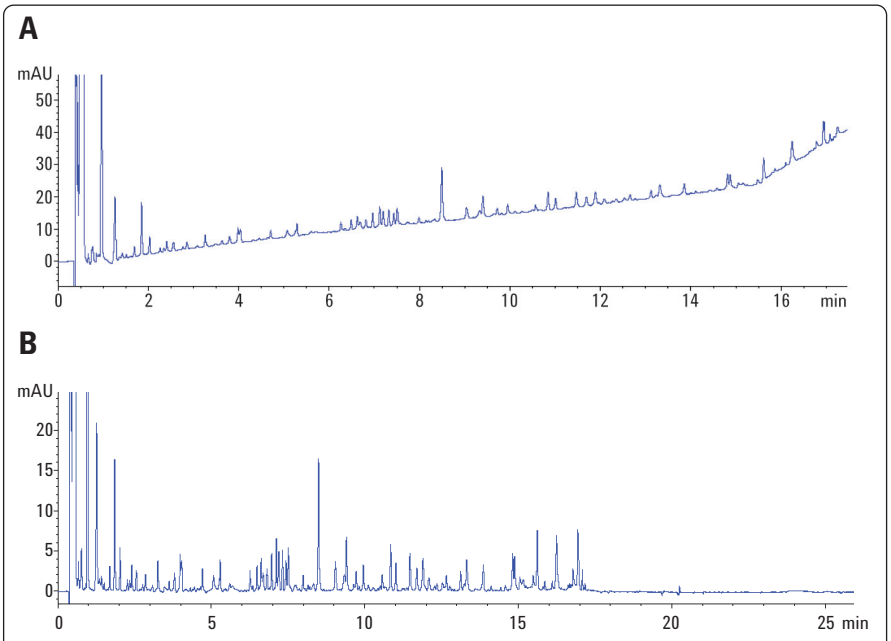
Configuration

Agilent 1290 Infinity LC system consisting of:

- Agilent 1290 Infinity Binary Pump with Integrated Vacuum Degasser (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1200 Diode Array Detector (G1315C)

Conclusion

The UHPLC method shortens separation time and improves peak capacity. It also enables faster re-equilibration after analysis. More details can be seen from the fingerprints. This method improves accuracy and precision of human factor IX analyses.



A:	Zoomed chromatography
B:	Fingerprint chromatography subtracted blank run
Condition:	0.1% (v/v) TFA-aqueous was used as the mobile phase A, ACN: water (80:20 v/v) which added 0.1% (v/v) TFA was used as the mobile phase B. 0.6 mL/min
Gradient:	0 min-0% B,6 min-15% B,15 min-35% B, 20 min-85% B, 24 min-100% B, 25 min-0% B, 26 min-0% B
Stop time:	26 min
Post time:	2 min
Column temperature:	45 °C
Column:	Agilent ZORBAX SB C8 RRHD (2.1 mm × 100 mm, 1.8 µm)
UV detection:	220 nm

Figure 1
Fingerprint chromatography of human factor IX.

www.agilent.com/chem/1290

June 1, 2010
Publication Number 5990-5991EN

Agilent Technologies