FlexFire
HPLC & UHPLC Columns

Insulin Analysis with UHPLC-MS

S. Horikiri

■ Introduction

Proteins, including small ones such as insulin, often require strong ion pairing agents (e.g. TFA) for optimal separation and peak shape. However, strong ion pairs often do not dissociate during the ionization step of MS detection, leading to charge masking and lowered sensitivity. Formic acid, a weak ion pairing agent, offers much higher sensitivity with MS detection but at the expense of broader peaks during LC separation. Here we applied Develosil FlexFire C18, 1.6 µm (2 x 50 mm) to the analysis of insulin with both strong (DFA, TFA) and weak (formic acid) ion pairing agents to see how they increase the height of the insulin peak.

■ Method and Results

0.1% Formic acid is a simple mobile phase composition. It does not require pH adjustment which prevents mistakes in the process of mobile phase preparation. To detect small to large proteins with LC-MS, 0.1% formic acid is the optimal mobile phase. Small peptides do not normally present resolution issues, but can exhibit impaired peak shapes. Larger molecules however, tend to show broader peak shapes with more tailing issues. We tested 3 different brands of columns as shown in

Fig.1. FlexFire showed the highest retention among the three when using a simple mobile phase of 0.1% formic acid, as well as partially resolving an impurity peak. Column A and B were unable to separate the impurity. FlexFire with 0.1% formic acid showed sufficient separation for the MS detection without using a strong ion pairing agent.

Fig. 2 shows the chromatograms obtained using different mobile phase modifiers. 0.1% Formic acid was replaced with 0.1% acetic acid, 0.1% DFA or 0.1% TFA. DFA and TFA helped to improve peak shape and the separation of the impurity. However, these conditions usually greatly decrease sensitivity with MS detection.
We used low protein binding vials to handle insulin samples because with normal glass vials we observed significantly lower peak heights due to adsorption.

■ Analysis Condition:

Sample: Insulin human recombinant (M.W. = 5807.57)

Mobile phase: A) Water + 0.1%HCOOH
B) Acetonitrile + 0.1%HCOOH

Gradient:

Gradient				
min	mL/min	%A	%B	Curve
0.00	0.5	80	20	5
5.04	0.5	40	60	5
5.05	0.5	40	20	5

Temperature: 40C
Detection: UV 280 nm
Injection volume: 0.3 µL
System: Thermo Scientific Vanquish H
Mixer: 10 µL

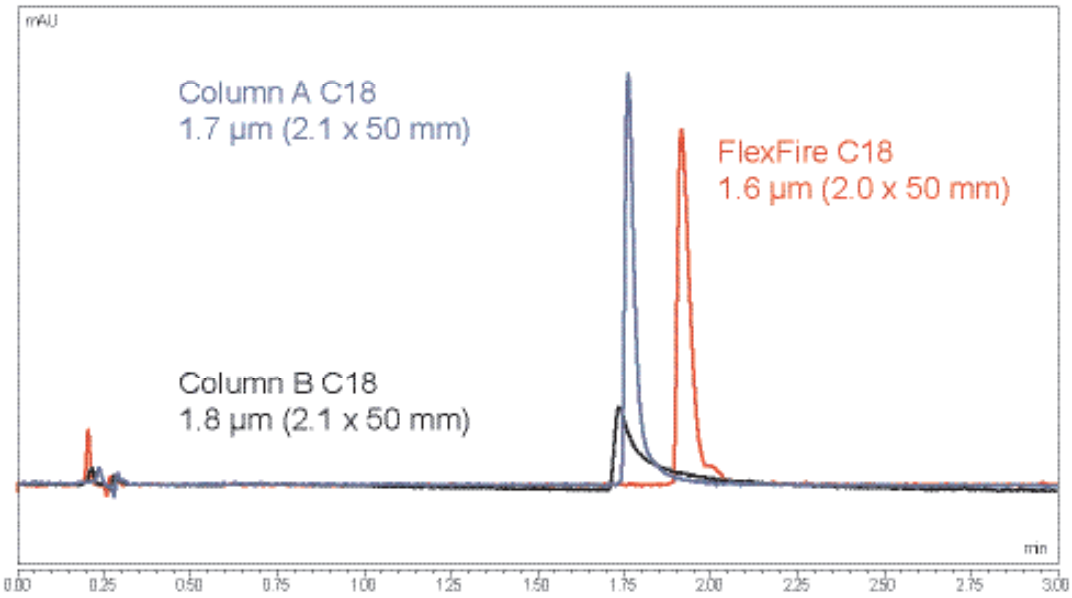

Fig 1. Insulin analysis in the mobile phase of 0.1% formic acid. (image attached)

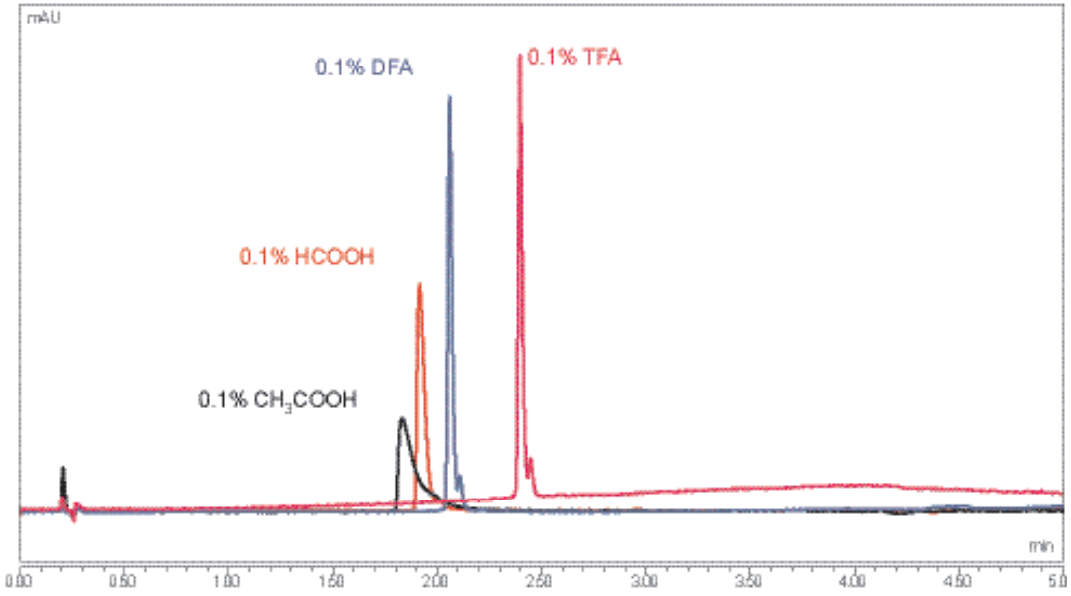

Fig 2. Insulin analysis in different mobile phase (image attached)

■ Order Information

Develosil UHPLC 1.6 μm Series

Size	C30	C18	C8	C1	HILIC
2.0 x 35 mm	201-I20035W	202-I20035W	203-I20035W	204-I20035W	205-I20035W
2.0 x 50 mm	201-I20050W	202-I20050W	203-I20050W	204-I20050W	205-I20050W
2.0 x 75 mm	201-I20075W	202-I20075W	203-I20075W	204-I20075W	205-I20075W
2.0 x 100 mm	201-I20100W	202-I20100W	203-I20100W	204-I20100W	205-I20100W
2.0 x 150 mm	201-I20150W	202-I20150W	203-I20150W	204-I20150W	205-I20150W

Develosil UHPLC 1.6 μm Metal-free Series

Size	C30	C18	C8	C1	HILIC
2.0 x 35 mm	201-I20035MFW	202-I20035MFW	203-I20035MFW	204-I20035MFW	205-I20035MFW
2.0 x 50 mm	201-I20050MFW	202-I20050MFW	203-I20050MFW	204-I20050MFW	205-I20050MFW
2.0 x 75 mm	201-I20075MFW	202-I20075MFW	203-I20075MFW	204-I20075MFW	205-I20075MFW
2.0 x 100 mm	201-I20100MFW	202-I20100MFW	203-I20100MFW	204-I20100MFW	205-I20100MFW
2.0 x 150 mm	201-I20150MFW	202-I20150MFW	203-I20150MFW	204-I20150MFW	205-I20150MFW

■ Contact Us

Develosil®

NOMURA CHEMICAL

Develosil USA

10060 Carroll Canyon Rd. Ste. 100
San Diego, CA 92131

Phone: 858-800-2433

Web: https://develosil.us/

Nomura Chemical Co., Ltd.

15, Hinode-cho, Set, 489-0004, Japan

Tel: +81-561-48-1853

Fax: +81-561-48-1434

E-mail: info@develosil.net