

Insulin Analysis with UHPLC-MS

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

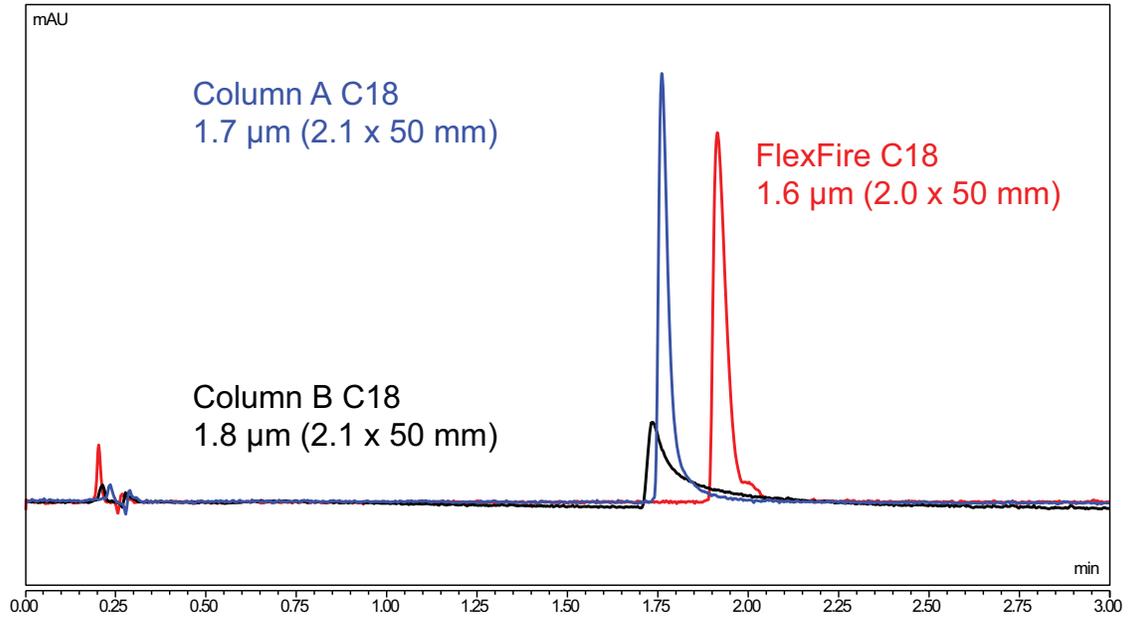


Fig 1. Insulin analysis in the mobile phase of 0.1% formic acid.

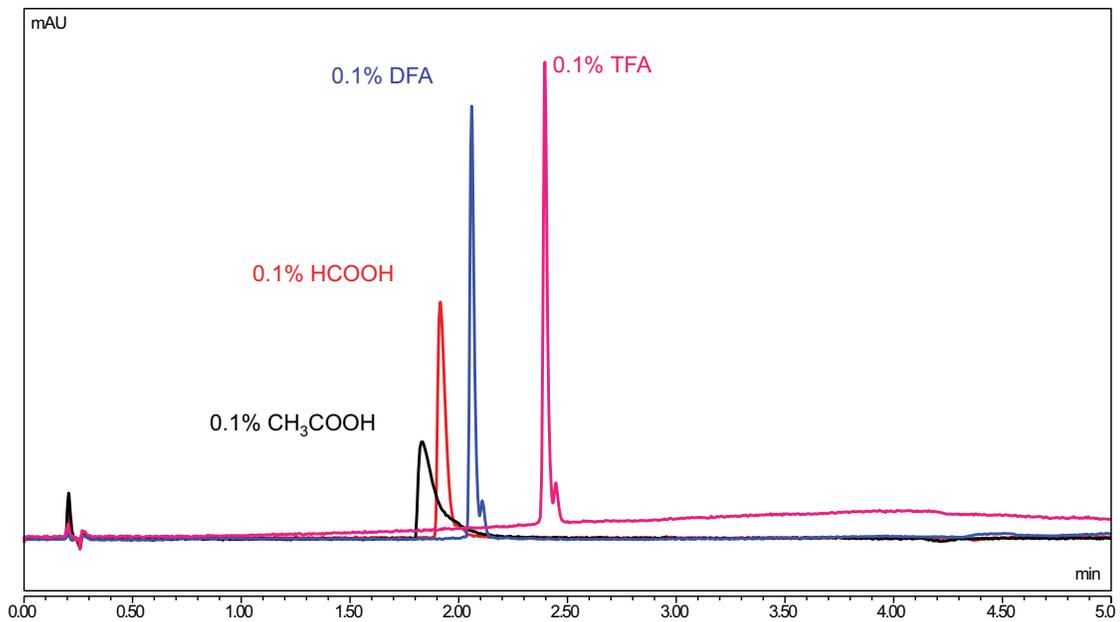


Fig 2. Insulin analysis in different mobile phase.

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