Separation of Peptides Using a Core Enhanced Technology Accucore HPLC Column

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Abstract
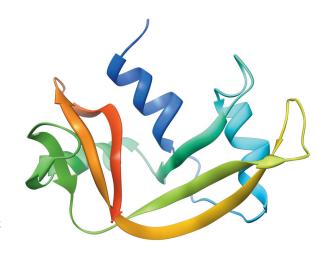
This application note demonstrates the use of Thermo Scientific Accucore C18 for the separation of peptides with excellent resolution between low and high molecular weight peptides in under four minutes.

Introduction

Accucore™ C18 columns use Core Enhanced Technology to offer high efficiencies to separate low and high molecular weight peptides. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimised phase bonding creates a series of high coverage, robust phases. The carbon loading of Accucore™ C18 provides high retention of non-polar analytes via a predominantly hydrophobic interaction mechanism, enabling the separation of a broad range of analytes. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Sample Preparation

A 50 µg/mL peptide standard mixture was prepared in water (refer to Table 1)

Thermo Scientific Column	Part Number
Accucore C18 2.6 µm 100 x 2.1 mm Measured pressure: 242 bar	17126-102130

Thermo Scientific Accela HPLC/UHPLC

Column temperature	40 °C
Injection volume	2 µL
Flow rate	0.50 mL/min
PDA/UV detection	230 nm

Mobile Phase

A: 0.1% TFA Water

B: 0.1% TFA Far UV Acetonitrile

Gradient:

Time/min	A%	B%
0.01	90	10
6.00	30	70

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade Far UV Acetonitrile	268260025
Trifluoroacetic Acid, Fisher Scientific	T/3258/PB05
NSC Mass Spec Certified 2 mL clear vial with PTFE silicone cap	MSCERT4000-34W

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Results

Figure 1 shows the chromatogram of the peptides with varying molecular weights separated on an Accucore C18 2.6 µm 100 x 2.1 mm column in under 3.5 minutes. The analysis demonstrates this column can provide baseline resolution of peptides with a wide range of molecular weights, from Gly-L-Tyr (MW 238) to Ribonuclease (MW 13700).
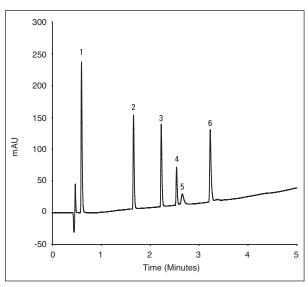
Conclusions

The separation of peptides was successfully achieved on an Accucore C18 column, therefore, providing an excellent choice for the analysis of these peptides, giving an excellent baseline resolution between high and low molecular weight peptides.

Figure 1: Chromatogram of peptides separated on the Accucore C18 2.6 µm 100 x 2.1 mm column

	Compounds	MW	t_r/min
1	Glycyl-L-Tyrosine	238	0.60
2	Val-Tyr-Val	380	1.66
3	Methionine Enkephalin	574	2.23
4	Leucine Enkephalin	556	2.54
5	Ribonuclease	13700	2.66
6	Insulin	5733	3.23

Table 1: The analysis of peptides on the Accucore C18 2.6 µm 100 x 2.1 mm column

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